GUSTATORY AND OLFACTORY FEEDING RESPONCES IN JAPANESE KOI CARP (*CYPRINUS CARPIO*)

BY

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Declaration

I, the undersigned, herby declare that the work contained in this assignment is my own original work and that I have not previously in its entirety or in part submitted it at any other university for a degree.

Philip Barnard

Date:

Abstract

Gustatory and Olfactory Feeding Responses in Japanese Koi Carp (*Cyprinus carpio)*

Chemo-attraction and –stimulation facilitate the initial location (olfactory response) and final consumption (gustatory response) of food in the feeding process of fish. Chemo-attractants or chemo-stimulants is therefore generally included in feeds for especially slow-feeding species to help reduce water fouling and to promote feed efficiency and growth rate through improved feed intake. Considering this, a study was performed to evaluate the attraction and stimulation potential of selected cereals and free amino acids in diets for Japanese koi carp (*Cyprinus carpio*). Results are presented on the comparative evaluation of five cereals (maize, sorghum, wheat, rye and triticale), raw and cooked forms of maize and concentrations of betaine and selected free amino acids (alanine, arginine, lysine and methionine), as well as their additive effect.

Opsomming

GUSTATORIESE EN OLFAKTORIESE RESPONS IN JAPANESE KARP

Chemo-aantreklikheid en -stimulasie fasiliteer die aanvanklike ligging (olfaktoriese respons) en die finale verbruik (gustatoriese repons) van kos in die voedingsproses van vis. Chemo-aantekkers of chemo-stimulante word dus gewoonlik ingesluit in voer veral vir spesies wat stadig vreet, om sodoende water besoedeling te help verhoed en om voeding doeltreffendheid en die groeikoers deur vehoogte voer inname te bevorder. Met dit in gedagte is hierdie studie onderneem om die aantrekking- en stimulasie potensiaal van geselekteerde grane en vry ominosure in die diet van Japanese koi karp (*Cyprinus carpioi)* te evalueer. Die studie toon die uitslag van 'n vergelykende evaluasie van vyf grane(mielies, sorgum, koring, rog,en tritikale) en die rou en gekookte vorms van mielies en van konsentrasies van betanien en gesekteerde vry aminosure (alanien, arginien, lisien en methionien), asook hulle byvogsel verhouding.

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Chapter 1

Gustatory and Olfactory Feeding Responses in Fish

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Introduction

Feeding in fish, as in other groups of animals, is an important function of life, and is a result of processes that are associated with searching, aiming, accepting, seizing, oral processing and evaluation of the quality of food objects. Swallowing, digestion, absorption and assimilation follow these processes. The success of these tasks, directly related to the satisfaction of energy requirements, is responsible for the rate of growth and maturation of fish and their resistance to the impact of unfavourable factors.

Many sensory systems contribute to fish feeding behaviour, but their role and significance may profoundly differ at different phases of the feeding behaviour. Chemo-attraction and chemo-stimulation facilitate the initial location (olfactory response) and final consumption (gustatory response) of food in the feeding process of fish. Chemo-attractants or chemo-stimulants are therefore generally included in feeds to help reduce water spoiling, promote feed efficiency and growth rate through improved feed intake.

Classification of taste substances & Feeding mechanisms employed by fish

Chemical substances can be divided into several categories or types, according to their effects on the feeding behaviour of fish. There are distinctions between the substances that have effects on the oral (gustatory) and extraoral (olfactory) taste systems. These systems play different roles in fish feeding behaviour (Atema 1980; Pavlov and Kasumyan, 1990) and differ in their functional characteristics (Marui and Caprio, 1992; Kasumyan 1999a). The presence or absence of these compounds in the diet determines whether a food item is grasped or ignored, is eaten or rejected, and to some extent, how much of the food is consumed.

Chemo-attractants effecting feeding behaviour in fish are classified according to Lindstedt, (1971); Mackie, (1982); Mearns et al., (1987); Sakata, (1989) as follows:

- 'Incitants' are substances that induce capture of the food item via the extraoral taste system. There are a number of different behaviours that can presumably be evoked by incitants like: suction, grasping, snapping, biting, tearing or pinching.
- 'Suppressants' are substances that decrease the rate of grasping food items. Like incitants, suppressants are mediated by the extra oral gustatory system and control the rate of grasping food items.
- 'Stimulants' are substances characterised by high ingestion rate. This behaviour is evoked by the oral taste system. Stimulants promote fish feeding. Usually, fish swallow the food items containing stimulants at first capture. This behaviour is mediated by the oral gustatory system.

- 'Deterrents' are substances that make the fish abandon food intake and evoke food rejection. Deterrents are characterised by high rejection and low ingestion of food items that are caught. Often, fish feeding motivation is decreased for a short period after a fish has tested a food item that contains a deterrent. Usually, the retention time of food items that contain a deterrent substance is short and the fish does not attempt to re-catch the food item. This behaviour is mediated by the oral gustatory system.
- 'Enhancers' or potentiators are substances that, although not feeding stimulants, accentuate the flavour of the food and cause fish to increase their consumption of flavoured food. In some cases, however, stimulants may be ineffective as feeding enhancers. This behaviour is evoked by the oral taste system.

As will be shown, a single substance might have different effects on the taste behaviour of the same fish and can be both incitant and deterrent, or incitant and stimulant. A number of substances do not induce any effects on fish behaviour either via the extraoral or the oral taste systems and are classed as 'indifferent' substances

In the Location and identification of food, fish may rely on one or a combination of the following sense stimuli.

- Visual detection

Visual identification, eye sight can play an important role in location and identification of food. Visual location and identification decreases at night fall or when water body is murky, as is the case with water that has a high level of suspended solids (dark brown water).

- Electro detection

Electro-sensory detection in fish is achieved with the use of small electro pulses which are emitted by the fish itself, these pulses are then interoperated as they reach the fish again, after reflection by the prey or food (much like a bat navigates itself in a dark cave using sonar). This form of detection can improve information on location, size, shape and quality of objects in the predator’s immediate vicinity (Kalmijm, 1974). In fish that possess well-developed eyes and electro-sensory systems can compensate to allow food location under poor visual conditions (dark brown water).

- Mechano-reception

Fish use stimulus cues like sound and turbulence to locate food (Winn, 1964); this stimulus is registered by the mechano-receptors located on the lateral line. Fresh water bass and trout have a well developed lateral line and depend on this form of detection in the identification of possible prey and food.

- Chemo-reception

Fish locate foodstuff by following chemical signals produced by its respective prey or food, this is known as olfactory response. The final consumption of the food item is determined by the gustatory response of the fish towards the food item, this determines the palatability of that food item. Atema (1971), demonstrated that catfish (*Ictalurus spp*.) can locate distance chemical signals by taste alone, through their well developed sense organs located in their mouth cavity.

Olfactory Response in Fish

Olfactory (smell) receptors of animals have evolved a certain degree of compatibility with the external chemical environment which enables them to cope with a wide variety of naturally occurring compounds, consequently the spectra of chemicals detected by various animals would vary depending upon their surrounding environment. In fish, unlike terrestrial animals, olfaction takes place entirely in the aquatic environment (Harpaz et al., 1987). The carrier of stimulant chemical molecules is not air but water, thus chemicals that are detected olfactorily by fish need to be volatile, but must be soluble in water.

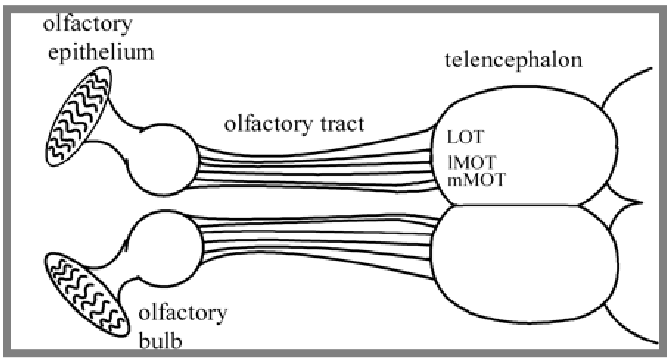

Figure 1: Olfactory Organ of the Common Carp (*Cyprinus carpio*) (Atema, 1980)

Taste buds are found in exceptionally large numbers on the external body surface of catfish, with the greatest density occurring on the barbells (Parker, 1910).

Various types of fish behaviour are mediated through olfaction. Some fish employ the sense of smell to find food; odours may be released by freshly killed, injured or already decaying organisms or the odour of a prey can be significant to a predator. In other fish species, the olfaction is especially important in migration and homing, the degree of alarm or aggression can be conveyed by olfaction (Atema, 1980).

Gustatory Response in Fish

The gustatory (taste) sensory system provides the final evaluation in the feeding process. It is well known from both scientific research and the anecdotes of fishermen that the taste properties of feeds or baits have a dramatic influence on food consumption of fish, growth rate and fishing success (Jones, 1980; Mackie & Mitchell, 1985; Takeda & Takii, 1992; Kasumyan, 1997).

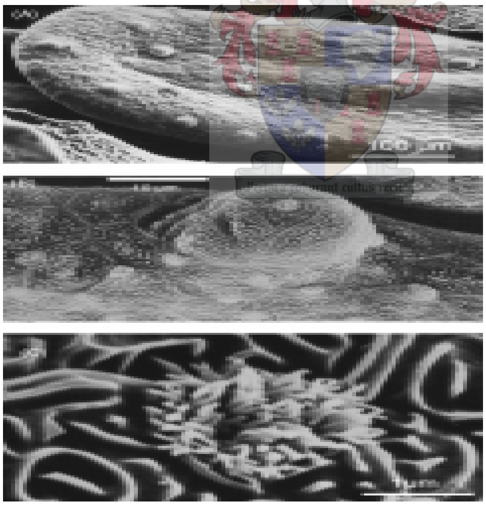

Figure 2: An electro micrograph representation of the peripheral organs belonging to the gustatory system, are the taste buds. Tongue tip of European eel larvae, (*Anguilla anguilla)* (Reutte *et al.*, 1974).

In fish, the taste buds are situated not only within the oral cavity, pharynx, oesophagus and gills, but may also occur on the lips, barbells, fins, and over the entire body surface in many species. In cyprinids, the density of oral taste buds is between 300 and 400 mm^2 in the palatal organ (Osse et al., 1997)

Using the free amino acids and their derivatives as taste substances, it has been found that there are rigid requirements of taste receptors for the molecular structure of the stimulus molecules. For example, gustatory responses to amino acids are highly stereo-specific: the l-isomers of amino acids have, for most species studied, been shown to be more stimulatory than its d-enantiomers. Unsubstituted, l--amino acids are usually the most efficient compounds for the gustatory system of fish.

Species related specificity to attractants

Evidence has accumulated showing that amino acids may play an important role as chemical signals in olfactory and gustatory response in fish, and the correlation between stimulatory effectiveness and molecular structure of amino acids has been suggested (Suzuki & Tucker, 1971). General findings so far indicate that, (1) only α-amino acids are highly stimulatory, (2) L-isomers are always more stimulatory than D-isomers and (3) the stimulatory effectiveness is not directly related to the essential amino acids. Neutral amino acids containing two or fewer carbon atoms, and having unbranched and uncharged side chains, are highly stimulatory (Satou, 1971). Acidic amino acids are poor gustatory stimuli, and basic amino acids are quite variable, depending upon the fish species. A synergistic effect was observed for mixtures of some amino acids in the gustatory system of several fish species (Hidaka et al., 1976; Yoshii et al., 1979) and, in addition, electro-physiological responses to some amino acids are enhanced by the presence of another amino acid in cross-adaptation experiments (Marui & Kiyohara, 1987).

Table 1: Species related specificity to attractants and their inclusion rate.

Specie	Attractant	Inclusion rate	Reference
Atlantic salmon	L-Alanine	3.2 x 10^{-7}M	Sutterlin & Sutterlin, 1971
(*Salmo salar*)	L-Methionine	3.2 x 10^{-7}M	Sutterlin & Sutterlin, 1971
Carp	L-Glutamine	10^{-6}M	Goh & Tamura, 1978
(*Cyprinus carpio*)	Betaine hydrochloride	10^{-4}M	Yasumasa & Tamura, 1979
	L-Lysine	10^{-4}M	Goh & Tamura, 1978
	L-Methionine	10^{-4}M	Goh & Tamura, 1978
	L-Arginine	10^{-4}M	Satou, 1971
	L-Alanine	10^{-4}M	Satou, 1971
Catfish	Alanine	3.2 x 10^{-7}M	Suzuki & Tucker, 1971
(*Ictalurus catus*)	Glutathione		Suzuki & Tucker, 1971
	L-Glutamine		Fijiya & Bardach, 1966

	L-serine		Fijiya & Bardach, 1966
	Cysteine		Bardach et al., 1967
	L-alanine > L-arginine > L-serine > L-α-aminobutryc acid > L-glutamine > D-alanine > glycine	10^{-9}M - 10^{-11}M	Caprio, 1975
Chinook Salmon (*Oncorhynchus ts hawytscha*)	Glycine > Betain > Alanine	0.01M	Hughes, 1991
Crab	Betaine + Glutamine +		
Conger eel (*Conger myriaster*)	L-glutamine	Below 10^{-8}M & 10^{-9}M Solution	Goh et al., 1978
Dover sole (*Solea solea*)	Betaine		Mackie et al., 1980
	Glycine		Mackie et al., 1980
	Glycine = L-Alanine >		Mackie, 1982
	L-Lysine>		
	L-Phenylalanine >		
	L-Glutamic acid		
Eel (*Angiulla anguillato*)	D-glutamine	10^{-7}M	Sola & Tongiorgi, 1998
	D-glutamic acid	10^{-7}M	Sola & Tongiorgi, 1998
	D-alanine	10^{-9}M	Sola & Tongiorgi, 1998
	B-alanine	10^{-7}M	So la & Tongiorgi, 1998
Eel (*Angiulla anguilla*)	L-Alanine	285mg/100g feed	Kamstra *et al.*, 1991; Heinsbroek & Krueger, 1992.
	Glycine	508mg/100g	
	L-Histidine	39mg/100g	
	L-Proline	217mg/100g	

Flounder (*Pseudopleuronectes americanus*)	Glycine> Alanine> L-Methionine	10^{-3}	Sutterlin, 1975
Mummichog (*Fundulis heteroclitus*)	GABA> L-Alanine> L-Histidine> Glycine> β-Alanine	10^{-4}	Sutterlin, 1975
Octopus (*Octopus maya*)	ATP	10^{-5}M	Lee, P.G., 1992
	L-alanine	10^{-4}M	Lee, P.G., 1992
Pigfish (*Orthopristis*)	Betaine	C_{150} of ca 10^{-5}M	Carr et al., 1977
			Carr, 1976
Pinfish (*Logodon Rhomboides*)	Alanine		Mackie et al., 1980
	Betaine*		Carr & Chaney, 1976
	Aspartic acid		Carr & Chaney, 1976
	Glycine		Carr & Chaney, 1976
	Glutamine		Carr & Chaney, 1976
	Isoleucine		Carr & Chaney, 1976
	Phenylalanine		Carr & Chaney, 1976
Puffer (*Fuga pardalis*)	Glycine, L-glutamine, L-aspartic acid, L-isoleucine, L-phenylalanine & betaine		Carr & Chaney, 1979
	Betaine		Hidaka et al., 1978
	Glycine		Hidaka et al., 1978
	Proline		Hidaka et al., 1978

Rainbow trout (*Salmo gairdneri*)	L-Glutamine > L-Methionine > L-Leucine > Homoserine > L-Asparagine > L-Alanine > L-Cystine > L-Cysteine > Glycine > L-Serine > L-Histidine	10^{-4} M (Threshold: 10^{-7} – 10^{-8}M)	Hara, 1973
Red drum (Sciaenops ocellat us)	Glycine	2g/100g feed	McGoogan & Gatlin III, 1997
Sea bream (*Chrysophrys major*)	Serine		Hidaka et al., 1978
	Betaine		Ina & Matsui, 1980
	Alanine		Ina & Matsui, 1980
	Glycine		Ina & Matsui, 1980
	Valine		Ina & Matsui, 1980
	Alanine		Kolkovski et al., 1997
	Glycine		Kolkovski et al., 1997
	Arginine		Kolkovski et al., 1997
	Betaine		Kolkovski et al., 1997
	L-glutamine	10^{-7}M Solution	Goh et al, 1978
Shrimp/ prawn (*M.rosenbergii*)	Betaine HCL	Solution 6 ml solution at concentration of 10^{-3} M, 2h after feeding	Harpaz, 1997
Shrimp/ prawn (*P.monodon*)	AMP (adenosinemonop hosph.)	>10^{-2}M Solution	Coman et al., 1996

	Betaine* (mixture of Betaine, Glutamine & Taurine showed best attractability)	10^{-6}M Solution	Coman et al., 1996
	Glutamine*	10^{-7}M Solution	Coman et al., 1996
	Taurine*	10^{-6}M Solution	Coman et al., 1996
	Alanine	10^{-5}M Solution	Coman et al., 1996
	Arginine	10^{-5}M Solution	Coman et al., 1996
	Glycine	10^{-4}M Solution	Coman et al., 1996
	Isoleucine	10^{-5}M Solution	Coman et al., 1996
	Serine	10^{-4}M Solution	Coman et al., 1996
Silverside (*Menidia menidia*)	L-Alanine> L-Methionine> β-Alanine> L-Threonine	10^{-4}	Sutterlin, 1975
Snook (*C undecimalis*)	L-Alanine	0.01M Solution in 3% agar matrix	Borquez et al., 1998
	L-Leucine*	0.01M Solution in 3% agar matrix	Borquez et al., 1998
	L-Glutamic acid*	0.01M Solution in 3% agar matrix	Borquez et al., 1998
	Glycine*[1]	0.01M Solution in 3% agar matrix	Borquez et al., 1998
	L-Proline*	0.01M Solution in 3% agar matrix	Borquez et al., 1998
	L-Isoleucine*	0.01M Solution in 3% agar matrix	Borquez et al., 1998

	L-Histidine	0.01M Solution in 3% agar matrix	Borquez et al., 1998
	L-Serine	0.01M Solution in 3% agar matrix	Borquez et al., 1998
	L-Lysine	0.01M Solution in 3% agar matrix	Borquez et al., 1998
	L-Arginine*	0.01M Solution in 3% agar matrix	Borquez et al., 1998
	Inosine-5-triphosphate	0.01M Solution in 3% agar matrix	Borquez et al., 1998
	Uridine	0.01M Solution in 3% agar matrix	Borquez et al., 1998
Striped bass *(Morone saxatileis)*	Serine	0.6%	Papatryphon & Soares, 2001
	Alanine	0.4%	
	Betaine	0.4%	
	Inosine-5'-monophosphate	0.2%	
Tilapia (*T. zillii*)	Glutamic acid>	0.01M Solution	Johnsen & Adams, 1986
	Aspartic acid=	0.01M Solution	Johnsen & Adams, 1986
	Serine=	0.01M Solution	Johnsen & Adams, 1986
	Alanine=	0.01M Solution	Johnsen & Adams, 1986
	Lysine	0.01M Solution	Johnsen & Adams, 1986
Turbot *(Scopthalmus maximus)*	Inosine	9.5×10^{-7}	Mackie & Adron, 1978

Relevance of Attractants to Aquaculture

The farming and husbandry of fish demand the adoption of intensively managed production systems, where feed management practices play an important role and requires serious research efforts for the generation of appropriate technology (Cyrino and Kubitza, 2003).

Supplementation of artificial, dry diets with attractants (feeding stimulants) can increase acceptability, and consequently the consumption of low palatability diets by fry and fingerlings, which can increase growth rate and productivity. This practice can also reduce feeding time and feed waste, while improving water quality and environmental safety (Lee and Mayer, 1996).

Food flavour is mediated chemically by substances inherent to the food, and it is affected by the chemo-sensitivity of the species (Adams et al., 1988). Therefore, mixtures of attractants are more effective than individual components (Kubitza, 1995). L-amino acids are known to mediate stimulatory responses in fish fish, particularly the neutral amino acids, organic, aminated bases and nucleotides are also involved in the stimulatory response of fish (Hughes, 1991).

Slow feeding aquatic life forms, such as abalone and crustaceans, orientates themselves towards the food source using their chemo-attractive properties. Adding amino acids to their food source will stimulate the chemo-sensory system and improve productivity (Hopfield, 1982).

Ecological considerations may require that substitution of fish meal by plant protein becomes a mandatory practice in fish diets in the near future, however fish feed containing high proportions of plant proteins may show reduced feed palatability due to anti-nutritional factors and unwanted dietary bulking agents (Reigh and Ellis, 1992; Gomes et al. 1995). Chemo-attractants or chemo-stimulants are therefore generally included in feeds containing high levels of plant proteins to help reduce water spoiling, promote feed efficiency and growth rate through improved feed intake (Papatryphon and Soares, 2000).

Relevance of Attractants to Recreational Fishing

Most, if not all, techniques for catching fish or other water-living animals such as shellfish employ bait or other attractants to which the desired species is attracted. In general, the attractant at the exterior portions of the bait is released more quickly than that of the interior portions. Accordingly, fast release can be obtained by concentrating the attractant at the exterior portions of the bait (http://hyperphysics.phy-astr.gsu.edu/hbase/kinetic/diffus.html). Conversely, a delayed release is obtained by placing the major amount of attractant on the interior of the bait. Attractant release rate can be varied somewhat by the amount of cellulose employed into the bait, with release rates generally increasing as the cellulose content decreases. Similarly, increasing the level of attractants in the water, generally decreases the rate of release (Sears et al., 1986). Cellulose pulp and the like can be employed to improve the physical strength of the bait if desired. Suitable attractants include liquefied fish or other marine products, fish oils, anise, amino acids or synthetic attractants.

Synthetic attractants: one especially useful additive is sodium chloride or other electrolytes, which may optionally, be employed in an amount from about 0 to about 15%, preferably from about 0 to about 5% of the combined weights of polymeric material and salt. The presence of salt or other electrolyte often increases the rate of diffusion of water into the artificial bait, thereby increasing the rate of release of attractants from the artificial bait (http://hyperphysics.phy-astr.gsu.edu/hbase/kinetic/diffus.html).

Bait containing high amounts of cellulose will therefore be more water stable, meaning it gradually releases the attractants over an effective period of time if the attractants are placed in the interior of the bait. Cold water should be used in preparing the bait to ensure that the attractants are not degraded or inactivated at elevated temperatures. The hydrated composition permits the fish attractant to diffuse slowly out into the surrounding water.

The ease at which prey or food is detected by a fish is a function of size, movement, shape and colour. (Ewert, 1970, 1974). The size of the prey or food source influences the probability of detection, this is to say that bigger objects are seen easier than smaller objects. To visual predators like trout and bass, movement is an important stimuli for detection and recognition of prey (Ewart, 1974; Smith, 1976), this is because the prey or food stuff can be seen more easily and its presence stimulates the lateral lines and mechano-receptors. Shape, plays a role in visual identification, shape takes the form of an object that resembles that of the predators natural food e.g. small fish, frogs, etc. (Herter, cited in Blaxter, 1970). Colour can be seen as light reflection of the upper surface of the food object. The contrast of the reflecting light and the background (dark water), play an important role in detection of food by fish. In general fish are much more effective at locating and exploiting high contrast as compared to low contrast food items (Sumner, 1934; Popham, 1942; Ware, 1973). Smallmouth bass (Micropterus sp.) are attracted in order of decreasing effect red, yellow, white, green, blue and black targets (Brown, 1937). Rainbow trout (Oncorhynchus mykiss) are attracted in order of decreasing effect against a greenish background, blue, red, black, orange, brown, yellow and dark green (Ginetz and Larkin, 1973).

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Chapter 2

Factors Affecting Olfactory and Gustatory Responses in Fish

Contents **pg.**

2.1 Introduction

Fish feeding motivation is affected by various environmental factors like water temperature, Pollutants such as heavy metals and low pH water. These factors may shift fish taste preferences by having a direct affect on olfactory and gustatory responses in Fish result in a decrease in food consumed.

2.2 Temperature

The ambient water temperature is one of the most potent abiotic variable affecting vital functions in exothermic animals like fish. Fluctuations in the water temperature occur with changes in season. Common carp restrict their activity in winter, seeing that the water's temperature has fallen below their optimal temperature range of 20 and 28 °C (Horváth et al., 1992). The behaviour of carp in winter (over-wintering) is often described as follows: the carp cluster together in large groups; they form a depression in the bottom of the pond and there pass the winter with minimal movement and feeding (Michaels, 1988). Carp refrain from excessive weight loss by feeding on benthic invertebrates found in the deeper regions of the pond. The depression areas and thus feeding area increases with the size of the pond (water body), therefore the larger the pond, the larger the feeding area the fish will utilize (Michaels, 1988; Horváth et al., 1992; Billard, 1999).

According to Adámek et al., (1990) temperature has an effect on taste preferences in fish. The grass carp shows different levels of relative food preference for the same objects in warm (20° C) and cold (13° C) water. In the 'warm' series the three amino acids, l-alanine, l-histidine and l-cysteine, were stimulants and caused a considerable increase in the number of pellets consumed. In the 'cold' series, the two amino acids, l-histidine and l-valine were stimulants.

2.3 Water Pollutants

Fish taste receptors are exposed to the environment and predisposed to the detrimental effects of water pollutants. It has been shown that many pollutants, especially heavy metals, affect fish taste reception by both damaging the taste buds and reducing the sensitivity to the taste stimuli (Brown et al., 1982; Klaprat et al., 1992).

Heavy metals pose a serious problem because they do not decompose or become eliminated from ecosystems (Gaal et al., 1985; Pujin et al., 1990). Among all the heavy metals, mercury (Hg) has become the subject of most concern due to its biomagnification (*Biomagnification* is the bioaccumulation of a substance up the food chain by transfer of residues of the substance in smaller organisms that are food for larger organisms in the chain) potential and toxic effects to aquatic organisms and human health. The sources of Hg pollution came from industrial effluents and sewage sludge relating to chloroalkali industry, the manufacture of electrical equipment and paint. Sediment is considered as the major source of contaminants for bottom dwelling and bottom feeding aquatic organisms (Farag et al., 1998; Staveland et al., 1993). Common carp stirs the benthic detritus into the water column, and preys on benthic invertebrates and aquatic insect larvae; this results in an accumulation of mercury (Hg), which in turn destroys the taste buds and reduces the sensitivity to the taste stimuli.

2.4 Water pH

Fish exposed to short-time low pH levels can induce drastic changes in the ability of the fish to respond to taste substances (Kasumyan & Sidorov, 1995). The possibility remains that the pH dampens and/or enhances the effect of particular amino acids by influencing their binding kinetics with available gustatory cell receptor sites, and thereby changes their perceived intensity and/or quality. Results by Marui et al., (1983a) indicated that there is no direct relationship between pH and palatability. However, gustatory response to amino acids is pH dependent. In common carp, taste response magnitudes are constant above pH 6.0 (Marui et al., 1983b).

At pH below 7, the gustatory response magnitudes of both rainbow trout and common carp increase dramatically, but these changes were in parallel to the increased response to acidified natural water alone (Marui et al., 1983a,b). The latter response is probably because of an elevated CO_2 tension. The taste system of fish is highly sensitive to CO_2 (Yoshii et al., 1980).

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Chapter 3

The Evaluation of Five Different Grain Samples for Palatability in Japanese Koi Carp (*Cyprinus carpio*)

CONTENTS **pg**

Abstract

A comparative evaluation for palatability (taste) in Japanese koi carp *(Cyprinus carpio)* is determined on five selected cereals (maize, sorghum, wheat, rye and triticale). A significant difference ($p \leq 0.05$) in favour of maize to sorghum, rye and wheat exists after 10 minutes of the experiment; however there is no significant difference between maize and triticale. After 30 minutes of the experiment there is no significant difference ($p > 0.05$) between any of the five cereals.

Introduction

Palatability can be seen as the ingestion process that precedes digestion; the palatability of the food determines ultimately what nutrients are available for digestion (Lee and Mayers, 1997). The poorer growth commonly observed in fish, fed feed containing high proportions of plant proteins may be related to a decrease in feed intake resulting from reduced feed palatability (Reigh and Ellis, 1992; Gomes et al., 1995). Warm water fish e.g. carp, catfish and tilapia tend to be omnivorous and their natural diet includes vegetable foods, because they secrete copious quantities of amylase which digests raw starch well (McCallum, 2000). Cold water fish such as trout are carnivorous, thus feed containing high levels of fibre and starch, which are unwanted dietary bulking agents, results in an increase in suspended solids in the water. The aim of the experiment was to evaluate the palatability efficiency of five different cereals (maize, sorghum, triticale, rye and wheat) for Japanese koi carp *(Cyprinus carpio)*.

Materials and Methods

Animals

The trials were performed at the warm water unit of the Division of Aquaculture of the University of Stellenbosch. Koi weighing approximately 60g was collected from a commercial carp hatchery (Cape Koi), randomly allocated to the experimental system (100 fish per experimental unit) and adapted for 14 days prior to the start of the experiment. During this time the fish were fed commercially available extruded feed pellets (AquaNutro tilapia grower) to apparent satiation.

System

A 10 x 25000 litre recirculation raceway system was used for the experimental system. Each of the raceways was supplied with a two-chamber test compartment according to Harada et al., (1996). The temperature of the system was maintained at 27° C for the duration of the trial.

Figure 1: Two-chamber test compartment and experimental system 10 x 25000 litre recirculation raceway system.

Dietary Treatments

Five test cereals (cooked -maize, -sorghum, -tritical, -rye, and -wheat) were mixed with water and knead into a dough ball. The treatments were replicated 5 times and the experiment replicated 3 times. Palatability as measured by the total fish count in the relevant compartments after ten and thirty minute intervals.

Statistical analysis

Results for differences in palatability were analysed for significant differences using one-way ANOVA and Tukey's Pairwise comparison test.

Results

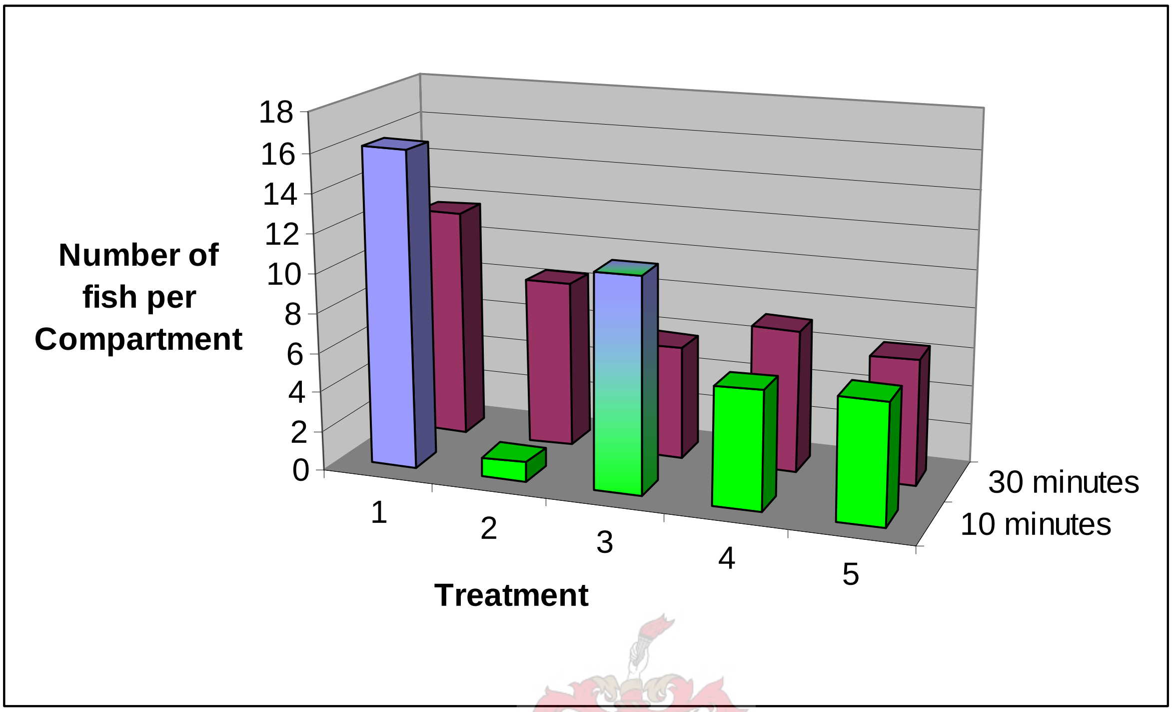

Figure 1. Influence of five different grains on Palatability in Japanese Koi Carp (*Cyprinus carpio*).

Table1. Means and standard errors for palatability of five different grains in Japanese Koi Carp (*Cyprinus carpio*)

Treatments	10 minutes	30 minutes
1. Maize	16.1± 3.42[a]	11.6 ± 3.48
2. Sorghum	0.63± 3.41[b]	8.49 ± 3.32
3. Triticale	10.9± 3.28[ab]	5.77 ± 3.34
4. Rye	5.97 ± 3.42[b]	7.21 ± 3.50
5. Wheat	6.14 ± 3.28[b]	6.37± 3.67

From table 1 it is evident that after 10 minutes of the experiment, there was a significant difference in favour of maize to sorghum, rye and wheat; however there was no significant difference between maize and triticale, maize showed a higher mean value of 16.1 than triticale of 10.9. After 30 minutes of the experiment, there is no significant difference ($p>0.05$) between any of the five treatments. Maize and triticale proved to be the most palatable ($p\leq 0.05$) cereal between the five samples.

Discussion

Maize proved to be the most palatable among the five grain types after 10 minutes. The high levels of palatability can be attributed to the fact that maize contains high levels of Methionine, in a L – configuration (Shen & Liu, 1992; Goh & Tamura, 1978). The decrease in feed intake of the other grains can be accredited to the influences of an essential amino acid imbalance, example sulphur amino acids (Medale et al., 1998), low phosphorus availability (Alarcon et al., 1990), and the metabolic effects of anti-nutritional factors (Alarcon et al., 1990). Anti-nutritional factors such as tannin found in sorghum, are not palatable resulting in a decrease in the ingestion rate of the food by the fish (Melcion & van der Poel, 1993). This anti-nutritional factor (tannin) could explain why sorghum has the lowest mean value showing a trend to be the least palatable of the five cereals after ten minutes.

After thirty minutes of the experimental unit there was no significant difference ($p\geq 0.05$) between any of the cereals. This effect can possibly be explained by a time concentration index. Tannins found in legumes and cereal give it characteristic bitter taste to fish reducing palatability, the amount of tannins in cereals is reduced by soaking the cereal (bait), the concentration index of the tannin is dependent on the duration of time that it is soaked. (Melcion & van der Poel, 1993). After thirty minutes of the experimental unit sorghum showed a trend to be the second most palatable after maize (mean value 8.49), this could be explained according to the time concentration index. Secondly the lack of significant difference could be explained to the fact that the fish may have escaped from the test compartments or even interred the compartment housing the less palatable cereal due to the lengthy time duration (30 minutes).

The ability of fish to detect and ingest a feed can be affected by physical characteristics such as pellet density (sinking rate), size (shape, diameter and length), colour (contrast), and texture (hardness) (Tucker, J.W., Jr., 1998). In general fish are much more effective at locating and exploiting high contrast as compared to low contrast food items (Sumner, 1934; Popham, 1942; Ware, 1973). The ingestion rate of less processed meals (cracked corn and sorghum) are consumed at a greater rate than the more processed carbohydrates example wheat and flour (Hardy, 1999). The five cereals have different colours and textures, these characteristic differences may have an effect on the resultant outcome. Maize proved to be the most palatable, to Japanese Koi Carp (*Cyprinus carpio*), after 10 minutes. However the palatability of the other cereals improved after 30 minutes of the experiment, due to the effect that the time concentration index may have on the anti-nutritional factors, and fish may have escaped from the test compartments due to the lengthy time duration.

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Chapter 4

The Effect of Maize gelatinization on Palatability in Japanese Koi Carp (*Cyprinus carpio*)

CONTENTS **pg.**

Abstract

A comparative evaluation for palatability (taste) in Japanese Koi Carp *(Cyprinus carpio)* is determined for raw and cooked forms of maize. After 10 minutes of experiment, there is no significant difference ($P \leq 0.05$) between the two treatments. After 20 minutes of the experiment, there is no significant difference ($P > 0.05$) between the two treatments. However, there was a trend towards the cooked maize, this can be seen is the higher mean values of cooked to that of raw maize.

Introduction

Palatability of maize can be improved by cooking it and effectively gelatinising the starch content (McCallum, 2000). Anti-nutritional factors and toxins present in plant ingredients, directly affect the fish palatability resulting in a decrease in the ingestion rate of the food (Melcion & van der Poel, 1993). Anti-nutritional factors can be classified according to their heat sensitivity, defined as the amount of heat necessary to inactivate the anti-nutritional factor without altering the biological value of the other components, thus the heat labile (lectins and protease inhibitors) or heat stable example tannins, oligosaccharides, saponins, phytate-oestrogens, phytate and alkaloids (Melcion & van der Poel, 1993). The aim of the experiment was to evaluate the palatability efficiency of raw (uncooked) and cooked maize for Japanese Koi Carp *(Cyprinus carpio)*.

Materials and Methods

Animals

The trial was performed at the warm water unit of the Division of Aquaculture of the University of Stellenbosch. Koi weighing approximately 60g were collected from a commercial carp hatchery (Cape Koi), randomly allocated to the experimental system (100 fish per experimental unit) and adapted for 14 days prior to the start of the experiment. During this time the fish were fed commercially available extruded feed pellets (AquaNutro tilapia grower) to apparent satiation.

System

A 10 x 25000 litre recirculation raceway system was used for the experimental system. Each of the raceways was supplied with a two-chamber test compartment according to Harada et al., (1996). The temperature of the system was maintained at 27° C for the duration of the trial.

Figure 1: Two-chamber test compartment and experimental system 10 x 25000 litre (m^3) recirculation raceway system.

Dietary Treatments

Two treatments 1. cooked and 2. raw (uncooked) maize, were replicated 5 times. The cooked maize was mixed with boiling water, while the uncooked, mixed with water at room temperature, both treatments were knead into a dough ball. Palatability as measured by the total fish count in the relevant compartments, after ten and twenty minute intervals.

Statistical analysis

Results for differences in palatability were analysed for significant differences using one-way ANOVA and Tukey's Pairwise comparison test.

Results

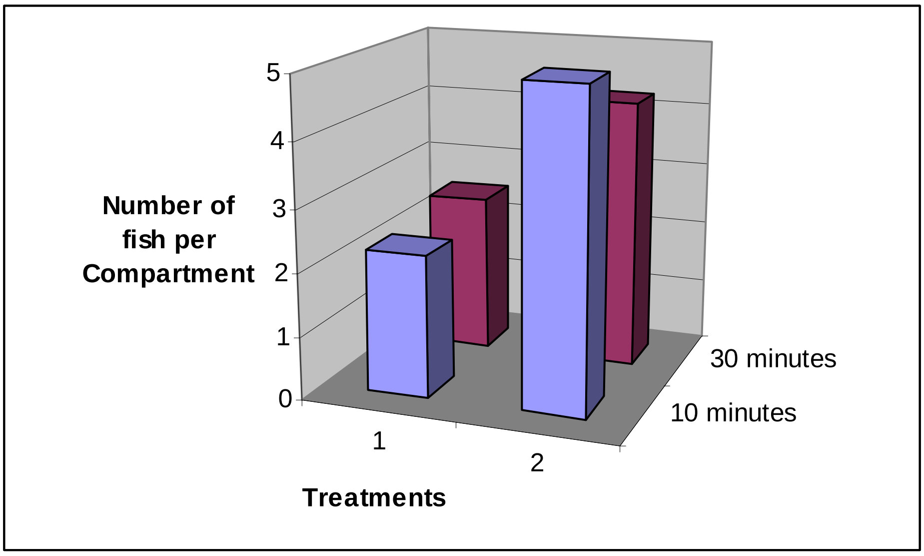

Figure 1. Influence of raw and cooked maize on Palatability in Japanese Koi Carp (*Cyprinus carpio*)

Table 1. Means and standard errors for palatability of raw and cooked maize in Japanese Koi Carp (*Cyprinus carpio*)

Treatments	10 minutes	30 minutes
1. Raw maize	2.25 ± 1.02	2.50 ± 1.21
2. Gelatinized maize	5.00 ± 1.02	4.25 ± 1.21

From table 1 it is evident that after 10 minutes of experiment, there was no significant difference (P>0.05) between the two treatments. After 20 minutes of the experiment, there was no significant difference (P >0.05) between the two treatments. However there was a trend towards the cooked maize, this can be seen is the higher mean values of cooked to that of raw maize.

Discussion

From the results there were no significant difference (P>0.05) between the cooked and uncooked maize during the experiment. The heat imparted by extrusion inflicts damage to the starch granule and renders it much more susceptible to amylase activity (He et al., 2002). Heat treatment decreases the activity of any heat labile anti-nutritional factors which can affect the palatability (Melcion & van der Poel 1993). Cooked and uncooked maize has no effect on the palatability as determined by Japanese Koi Carp (*Cyprinus carpio*).

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Chapter 5

The Evaluation of Maize/Sorghum Combination for Palatability in Japanese Koi Carp (*Cyprinus carpio*)

CONTENTS **pg**

Abstract

A comparative evaluation for palatability (taste) in Japanese Koi Carp *(Cyprinus carpio)* is determined on five combinations of maize and sorghum (maize 100%, maize/sorghum 75%/25%, maize/sorghum50%/50%, maize/sorghum 25%/75%, sorghum 100%). There was no significant difference (P ≤0.05) found between any of the five treatments during the experiment.

Introduction

A study undertaken by Osborne (2002), proved that the current grain sorghum starch content is at an average of 68 percent. Sorghum has no gluten, however through the gelatinization of its high starch content (68%) can perform as a binding agent (Slyer & Wing, 1999). Tannin, the anti-nutritional factor found in sorghum negatively affects the efficient use of sorghum based diets (Kondos & Foale, 1983). The Sudans sorghum used in the experiment typically has a tannin concentration of 10.0 g of this compound per kg of dry matter (Mehansho et al., 1985). The aim of this experiment was to determine the percentage sorghum that can be incorporated into maize to effectively improve the binding capacity, without decreasing palatability, because sorghum has a high tannin concentration which gives a characteristic bitter taste to fish (Melcion & van der Poel, 1993). Palatability efficiency of five combinations of maize and sorghum (maize 100%, maize/sorghum 75%/25%, maize/sorghum50%/50%, maize/sorghum 25%/75%, sorghum 100%) were tested in Japanese Koi Carp *(Cyprinus carpio).*

Materials and Methods

Animals

The trial was performed at the warm water unit of the Division of Aquaculture of the University of Stellenbosch. Koi weighing approximately 60g were collected from a commercial carp hatchery (Cape Koi), randomly allocated to the experimental system (100 fish per experimental unit) and adapted for 14 days prior to the start of the experiment. During this time the fish were fed commercially available extruded feed pellets (AquaNutro tilapia grower) to apparent satiation.

System

A 10 x 25000 litre recirculation raceway system was used for the experimental system. Each of the raceways was supplied with a two-chamber test compartment according to Harada et al., (1996). The temperature of the system was maintained at 27° C for the duration of the trial.

Figure 1: Two-chamber test compartment and experimental system 10 x 25000 litre (m^3) recirculation raceway system.

Dietary Treatments

The treatments consisted of 2 test cereals in combination with each other (maize 100%, maize/sorghum 75%/25%, maize/sorghum50%/50%, maize/sorghum 25%/75%, sorghum 100%) that were mixed with water at room temperature and knead into a dough ball. Treatments were replicated 5 times and the experiment replicated 3 times. Palatability as measured by the total fish count in the relevant compartments after 10 and 30 minute intervals.

Statistical analysis

Results for differences in palatability were analysed for significant differences using one-way ANOVA and Tukey’s pairwise comparison test.

Results

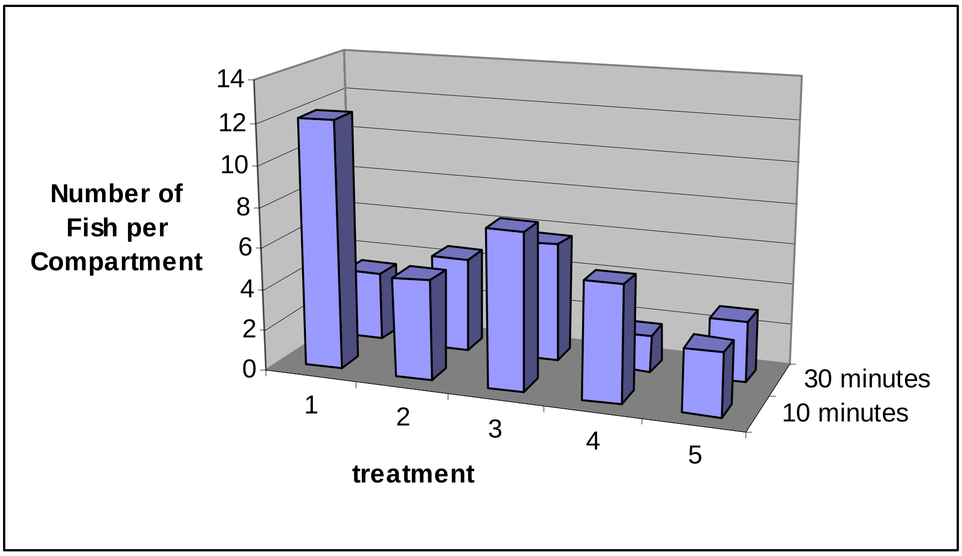

Figure 1. Influence of Maize (M)/Sorghum(S) combination on Palatability in Japanese Koi Carp (*Cyprinus carpio*)

Table 1. Means and standard errors for palatability of maize/sorghum combinations in Japanese Koi Carp (*Cyprinus carpio*)

Treatment	10 minutes	30 minutes
1. 100 M : 0 S	12.1 ± 2.18	3.40 ± 2.48
2. 75 M : 25 S	4.85 ± 2.18	4.60 ± 2.48
3. 50 M : 50 S	7.65 ± 2.18	5.80 ± 2.28
4. 25 M : 75 S	5.65 ± 2.18	1.80 ± 2.80
5. 0 M : 100 S	3.05 ± 2.18	3.00 ± 2.48

From table 1 it is evident that after 10 minutes of experiment, there was no significant difference ($P \leq 0.05$) between any of the five treatments, however maize at 100% showed the highest trend as food for the fish, this can be seen be the high mean value of 12.1. After 30 minutes of the experiment, there was no significant difference ($P \leq 0.05$) between any of the five treatments, however Maize/sorghum 50/50% shows the highest trend as food for the fish, this can be seen be the high mean value of 5.80.

Discussion

The results showed no significant difference between any of the five treatments used in the experiment. Sorghum has no gluten, however through the gelatinization of its high starch content (68%) can perform as a binding agent (Slyer, P. & Wing, S.L., 1999). This is the incorporation of 25% sorghum therefore allowing for the combination of 75/25 % maize/sorghum, will improve the binding capacity of the bait without having an adverse effect on the affinity of the maize as an effective food source for the fish due to the tannin found in sorghum.

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Chapter 6

The Effect of Betaine Hydrochloride Concentrations on Chemo-Attraction in Japanese Koi Carp (*Cyprinus carpio*)

CONTENTS **pg**

Abstract

A comparative evaluation for chemo attraction in Japanese Koi Carp *(Cyprinus carpio)* was determined on five combinations of betaine concentrations (10^{-2}, 10^{-4}, 10^{-6}, 10^{-8},10^{-10}). There was a significant difference ($p \leq 0.05$) between the treatments after the experiment. The concentration of 10^{-4} (15.66ppm) proved to be the most attractive to Japanese Koi Carp *(Cyprinus carpio).*

Introduction

Betaine is known to be a chemo attractant and feed stimulant for a number of aquatic fish and Crustaceae. In the genus *Crustacea*: sub-species *Palaemonetes pugio* betaine is a feed incident (Carr, 1978), an attractant in sub-species *Homarus americanus* (Carter & Steele, 1982), and a stimulant in sub-species *Macrobrachium rosenbergii* (Derby & Harpaz, 1988). Studies done by Konishi et al., (1966), showed betaine to be an attractant in the sea catfish (*Platosus anguillaris*). Betaine has been proven to be both a chemo-attractant and feed stimulant in the channel catfish (*Ictalurus catus*) and carp (*Cyprinus carpio*) as shown by Suzuki et al., (1971).

Studies by Yasumasa & Tamura (1979), showed Betaine hydrochloride, $C_{16}H_{24}N_2O_4 \cdot HCl$, to be more stimulating than pure betaine, due to an increase in HCl, as long as the pH remained above 3.5 (pH >3.5); below this ph (pH <3.5) the betaine hydrochloride will induce an inhibitory response. The aim of the experiment was to evaluate the chemo-attraction efficiency of five concentrations of betaine hydrochloride for Japanese Koi Carp *(Cyprinus carpio).*

Materials and Methods

Animals

The trial was performed at the warm water unit of the Division of Aquaculture of the University of Stellenbosch. Koi weighing approximately 60g was collected from a commercial carp hatchery (Cape Koi), randomly allocated to the experimental system (100 fish per experimental unit) and adapted for 14 days prior to the start of the experiment. During this time the fish were fed commercially available extruded feed pellets (AquaNutro tilapia grower) to apparent satiation.

System

A 10 x 25000 litre recirculation raceway system was used for the experimental system. Each of the raceways was supplied with a two-chamber test compartment according to Harada et al., (1996). The temperature of the system was maintained at 27° C for the duration of the trial.

Figure 1: Two-chamber test compartment and experimental system 10 x 25000 litre (m^3) recirculation raceway system.

Dietary Treatments

The concentrations for betaine hydrochloride (Batch number 2004050658, Warren Chem Specialities) was determined by making mixtures of the following concentrations 10^{-2}, 10^{-4}, 10^{-6}, 10^{-8},10^{-10}. First n = M/Mr was worked out by calculating the Mr from the molecular structure (Fig 1). These concentration were made up by mixing the M=nxMr with 1kg of maize/sorghum at (75%/25%), presented to the fish in a dough ball form, there was 5 treatments x 3 replicates in this experiment. The 10^{-10} is taken as the control because it is so diluted. Chemo-attraction as measured by total fish count in the relevant compartments after 10 and 30 minutes intervals.

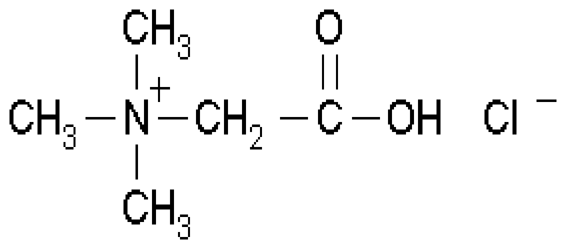

Fig 1: Betaine Hydrochloride Mr = 153.6

10^{-2} Concentration	10^{-4} Concentration	10^{-6} Concentration	10^{-8} Concentration:	10^{-10} Concentration:
10^{-2} concentration: N = 0.01. M = 0.01 x 156.6 = 1.566g into 1kg =1.566ppt = 1566ppm	10^{-4} concentration: N = 0.0001. M= 0.0001 x 156.6 = 0.01566 into 1kg =0.01566ppt =15.66ppm	10^{-6} concentration: N = 0.000001. M= 0.000001 x 156.6 = 0.0001566 into 1kg = 0.0001566ppt =0.1566ppm	10^{-8} concentration: N = 0.00000001. M=0.00000001x156.6 = 0.000001566 into 1kg = 0.000001566ppt =0.001566ppm	10^{-10} concentration: N = 0.0000000001. M= 0.0000000001 x 156.6 = 0.00000001566 into 1kg =0.00000001566ppt =0.00001566ppm

Statistical analysis

Results for differences in chemo-attraction were analysed for significant differences using one-way ANOVA and Tukey's pair wise comparison test.

Results

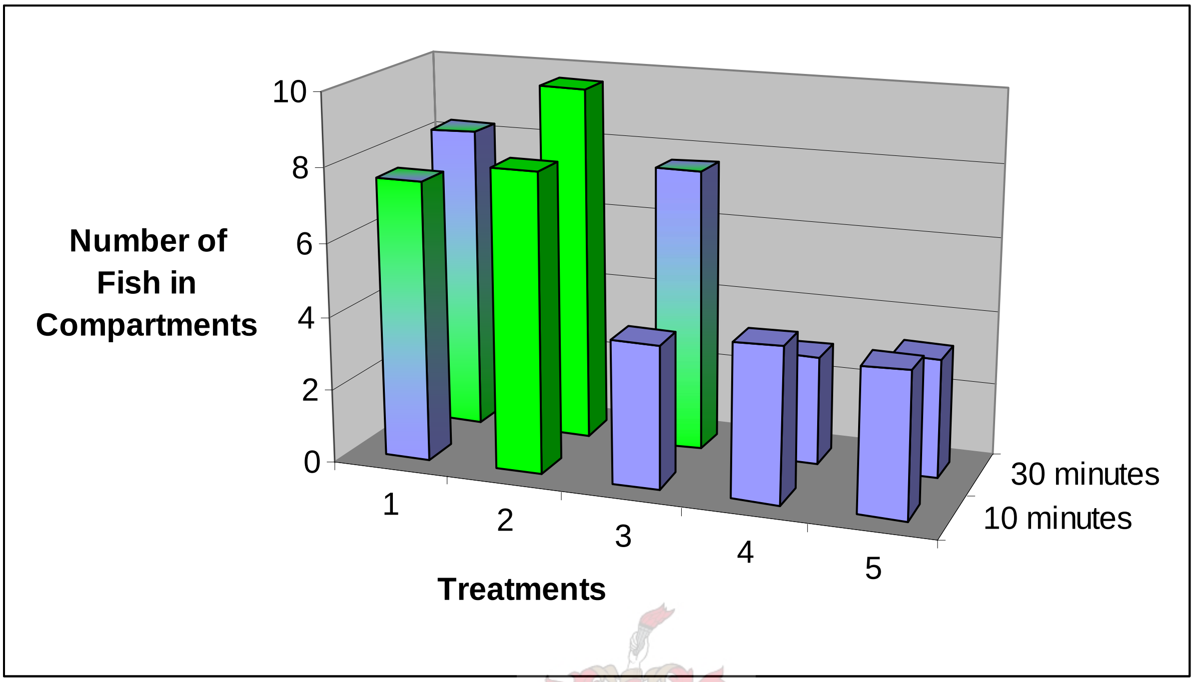

Figure 2. Influence of threshold concentrations on chemo attraction in Japanese Koi Carp (*Cyprinus carpio*)

Table 1. Means and standard errors for chemo attraction using betaine hydrochloride concentrations in Japanese Koi Carp (*Cyprinus carpio*)

Treatment	10 minutes	30 minutes
1. Betaine 1566ppm (10-2M)	7.63 ± 0.99^{ab}	8.29 ± 1.41^{ab}
2. Betaine 15.66ppm (10-4M)	8.11 ± 1.04^{a}	9.69 ± 1.48^{a}
3. Betaine 0.1566ppm (10-6M)	3.88 ± 1.02^{b}	7.68 ± 1.45^{ab}
4. Betaine 0.001566ppm (10-8M)	4.19 ± 1.01^{b}	2.94 ± 1.43^{b}
5. Betaine 0.00001566ppm (10-10M)	3.92 ± 0.96^{b}	3.25 ± 1.36^{b}

From table 1 after 10 minutes of the experiment, treatment 1 and treatment 2 showed a significant difference ($P \leq 0.05$) in relation to treatments 3, 4 and 5, there is no significant difference between treatments 1 and 2. After thirty minutes of the experiment, treatment 2 showed a significant difference ($P \leq 0.05$) in relation to treatments 4 and 5, there was no significant difference between treatments 1, 2 and 3.

Discussion

Receptors responsible for chemo-attraction and feed stimulation are found on the external body surface, maxilla, nostrils and buccal cavity of carp (Herrick, 1901; Atema, 1971; Davenport et al., 1979). Studies suggested that the density of flank taste buds on the external body surface of catfish is of a smaller density than those occurring on the barbells. Parker (1910), and Olmsted (1918), studying the olfactory reactions of the brown bullhead catfish, *Amiurus nebulosus*, concluded that the olfactory apparatus, is more sensitive than the organs of taste, this is the organ responsible for sensing food at a distance.

Tucker (1973), showed that there was a rapid decline of olfactory and gustatory receptors' sensitivity, which occurred with an increased holding time of the fish in the laboratory; he described this in relation to the receptor sites being progressively activated. This relation can be seen in the results, after ten minutes there was a significant difference ($p \leq 0.05$) between treatment 1 (1566 ppm) and treatment 2 (15.66 ppm) to the control, treatment 5 (0.00001566 ppm), however after thirty minutes only treatment 2 (15.66 ppm) is significant to the control, treatment 5. (0.00001566 ppm). It has been proposed that adaptation occurs when receptor sites are filled with the taste stimulus. Thus, the amount of cross adaptation between stimuli indicates the extent to which they compete for the same receptor sites (Beidler, 1962; Hellekant, 1969; Smith & Frank, 1972). An alternate proposal suggests that conformation changes of the taste receptor proteins, account for the post-excitatory depression rather than a long stimulus dissociation time (Smith and Bealer, 1976). In the response-concentration relation, as the concentration increases, there is an inverse relationship with time.

Concentrations above 1566 ppm were avoided because of possible injurious effects to the receptors (Herrick, 1904; Hoagland, 1933; Konishi et al., 1966; Biedenbach, 1971). Results from this trial indicate that concentration of 15.66ppm, betaine hydrochloride is potentially the best concentration for chemo-attraction and feed stimulation.

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Chapter 7

Evaluating the Effect of Amino Acids on Chemo-Attraction in Japanese Koi Carp (*Cyprinus carpio*)

CONTENTS **pg**

Abstract

A comparative evaluation for chemo-attraction in Japanese Koi Carp *(Cyprinus carpio)* was determined on four selected free amino acids (alanine, arginine, lysine, methionine, control). There was no significant difference ($P \leq 0.05$) after 10 minutes of the experiment, however results from this trial indicated significant differences ($P \leq 0.05$) after 30 minutes, between the four selected free amino acids.

Introduction

Chemo-reception is an important component of food detection in many aquatic animals (Harpaz et al., 1987). Behavioural studies have demonstrated repeatedly that amino acids and related compounds are potent feeding stimulants (Marui et al., 1992). General properties of fish feeding stimulants derived from animal tissue have been described by Carr (1982). These include:

1. low molecular weight (<1000)
2. non-volatile
3. nitrogenous, and
4. amphoteric.

Studies of feeding chemo-stimulants can provide information on chemo-sensory behaviour and physiology of the animals concerned, and may also detect attractants which can be incorporated into aquaculture feeds. This trial is mediated by two separate factors: chemical constituents of the food item and the animal's own chemo-sensitivity to those constituents. The chemical constituents of the food item determines the gustatory (taste) of the food item and will stimulate the fish to continue feeding (Heinen, 1980). The animal's own chemo-sensitivity towards the food item will orient itself towards the source of the chemical, known as olfaction (smell) (Heinen, 1980). According to Goh & Tamura (1978), L-lysine and L-methionine are seen as the best olfaction attractants for carp (*Cyprinus carpio*). Where Satou (1971), proved L-alanine and L-arginine to be the best gustatory feed stimulants. The aim of the experiment was to evaluate the chemo-attraction efficiency of four selected free amino acids in Japanese Koi Carp *(Cyprinus carpio).*

Materials and Methods

Animals

The trial was performed at the warm water unit of the Division of Aquaculture of the University of Stellenbosch. Koi weighing approximately 60g was collected from a commercial carp hatchery (Cape Koi), randomly allocated to the experimental system (100 fish per experimental unit) and adapted for 14 days prior to the start of the experiment. During this time the fish were fed commercially available extruded feed pellets (AquaNutro tilapia grower) to apparent satiation.

System

A 10 x 25000 litre recirculation raceway system was used for the experimental system. Each of the raceways was supplied with a two-chamber test compartment according to Harada et al., (1996). The temperature of the system was maintained at 27° C for the duration of the trial.

Figure 1: Two-chamber test compartment and experimental system 10 x 25000 litre (m^3) recirculation raceway system.

Dietary Treatments

Treatments of (alanine, arginine, lysine, methionine and control) with concentrations of 10^{-4}M (Coman et al., 1996) were presented to the fish in a dough ball form, consisting of a combination of maize/sorghum at (75/25%), there will be 5 treatments x 3 replicates. Chemo-attraction as measured by the total fish count in the relevant compartments after 10 and 30 minute intervals.

L- Alanine (batch no. 031104 Warren chem. Specialities) Mr = 89.09 n=0.0001 M=nxMr M=0.0001x89.09 = 0.008909ppt, therefore 8.909ppm.

L-Arginine (batch no. 04010389 Warren chem. specialities) Mr = 174.2
n =0.0001 M=0.01742ppt, therefore 17.42ppm.

L-Lysine (batch no. 030803 Warren chem. Specialities) Mr = 146.2
n = 0.0001 M=0.01462ppt, therefore 14.42ppm.

L-Methionine (batch no. 040702 Warren chem. Specialities) Mr = 149.2
n = 0.01 M=0.01492ppt, therefore 14.92ppm.

Statistical analysis

Results for differences in chemo-attraction were analysed for significant differences using one-way ANOVA and Tukey's pair wise comparison test.

Results

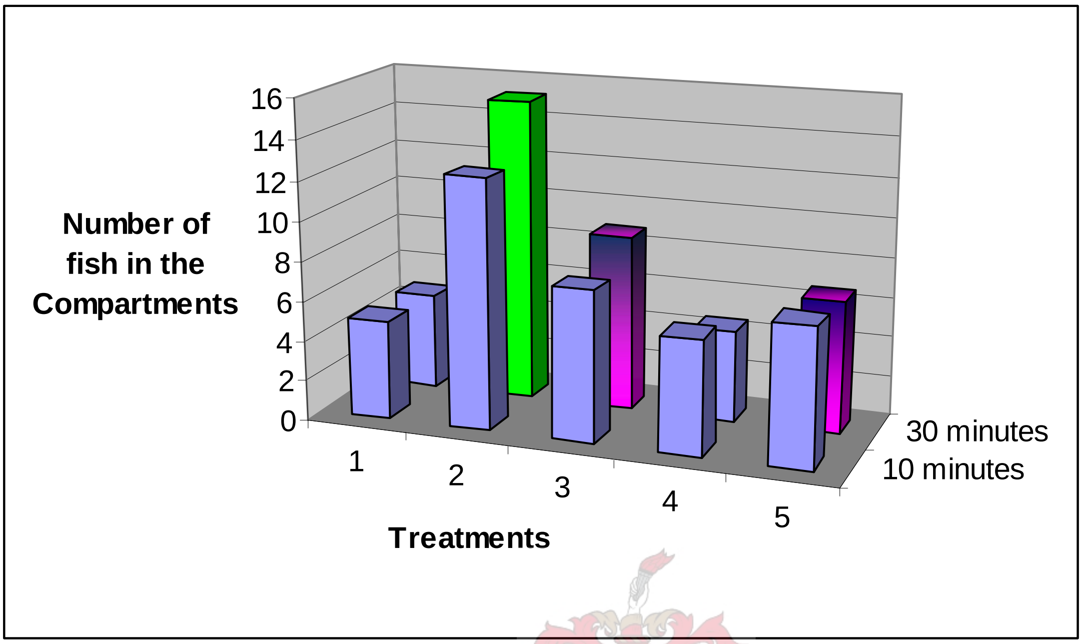

Figure 1. Influence of amino acids on chemo attraction in Japanese Koi Carp (*Cyprinus carpio*)

Table 1. . Means and standard errors for chemo attraction using free amino acids in Japanese Koi Carp (*Cyprinus carpio*)

Treatments	10 minutes	30 minutes
1. Arginine (17.42ppm)	4.95 ± 1.72	4.80 ± 0.72[c]
2. Alanine (8.909ppm)	12.5 ± 1.72	15.2 ± 0.72[a]
3. Lysine (14.42ppm)	7.55 ± 1.72	8.80 ± 0.72[b]
4. Methionine (14.92ppm)	5.75 ± 1.72	4.60 ± 0.72[c]
5. Control (maize/sorghum 75/25%)	6.95 ± 1.72	6.60 ± 0.72[b]

After ten minutes of the experiment, there was no significant difference between the treatments. After thirty minutes of the experiment, treatment 2 (L-alanine) had a significant difference ($P \leq 0.05$) from treatment 1 (L-arginine), 3 (L-lysine), 4 (L-methionine) and 5 (maize meal, control), and treatment 3 (L-lysine) and 5 (maize meal, control) show a significant difference ($P \leq 0.05$) from treatment 1 (L-arginine) and 4 (L-methionine). There was a greater trend towards treatment 3 (L-lysine) in relation to treatment 5 (maize meal, control), however this result is not significant.

Discussion

Parker (1910) concluded that the olfactory sense of catfish serves as a distance food-finding sense and is more sensitive than the organs of taste, this was later confirmed by Olmsted (1918). Electrophysiological evidence has revealed that olfactory receptors of catfish are highly sensitive to amino acids (Suzuki & Tucker, 1971). The gustatory sense, with its abundance of external taste buds (Herrick, 1901; Atema, 1971), has been found to be responsive to certain amino acids (Satou, 1971); two best stimulants are L-alanine and L-arginine, two best attractants are L-Lysine and L-methionine according to him for carp *(Cyprinus carpio)* (Goh and Tamura, 1978). Sato and Suzuki (1961) found that olfactory tract fibres in carp did not respond to NaCl, n-butyl and iso-amyl alcohols and their acetate esters. L-Alanine is one of the more prevalent amino acids in the tissues of worms, molluscs, crustaceans and teleosts (Campbell, J.W., 1970), which include the majority of food organisms for bottom feeders (Perry, W.G., 1969). Accordingly, L-lysine is in relatively large amounts in tissues of a variety of molluscs and crustaceans (Konosu, 1971; Wachtendonk and Kappler, 1977) and is one of the more abundant free amino acids in insect larvae (Corrigan, 1970). Results form this trial indicate that L-alanine is the best gustatory stimulant and L-lysine the olfactory attractant.

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Chapter 8

The Additive effect of Betaine Hydrochloride with Amino Acids on Chemo attraction in Japanese Koi Carp *(Cyprinus carpio)*

CONTENTS **pg**

Abstract

A comparative evaluation on the additive effect of betaine hydrochloride with amino acids on chemo attraction in Japanese Koi Carp *(Cyprinus carpio)* was determined. The four selected free amino acids (alanine, arginine, lysine and methionine) and betaine hydrochloride were tested at a concentration of 10^{-4}. There was no significant difference ($P \leq 0.05$) after 10 minutes of the experiment; however results from this trial indicated significant differences ($P \leq 0.05$) after 30 minutes, between the free amino acids and betaine.

Introduction

Amino acids appear to play a principal role in stimulating feeding in fish, however it is interesting to note that herbivore can be stimulated to feed by single amino acids (Carr, 1982; Mackie, 1982). In contrast to the results with Tilapia, some investigators (Hashimoto et al., 1968; Konosu et al., 1968; Adron & Mackie, 1978) working with carnivorous fish, report that whereas mixtures of amino acids are more effective feeding stimuli, than singly amino acids which often lacking in stimulatory efficiency. Previous research has indicated that mixtures containing betaine and amino acids are 9-16 times more effective as any of the other components used alone (Carr & Chaney, 1976; Carr et al., 1977; Carr, 1982). The aim of this experiment was to evaluate the additive effect of betaine hydrochloride with amino acids on chemo-attraction in Japanese Koi Carp *(Cyprinus carpio).*

Materials and Methods

Animals

The trial was performed at the warm water unit of the Division of Aquaculture of the University of Stellenbosch. Koi weighing approximately 60g was collected from a commercial carp hatchery (Cape Koi), randomly allocated to the experimental system (100 fish per experimental unit) and adapted for 14 days prior to the start of the experiment. During this time the fish were fed commercially available extruded feed pellets (AquaNutro tilapia grower) to apparent satiation.

System

A 10 x 25000 litre recirculation raceway system was used for the experimental system. Each of the raceways were supplied with a two-chamber test compartment according to Harada et al., (1996). The temperature of the system was maintained at 27° C for the duration of the trial.

Figure 1: Two-chamber test compartment and experimental system 10 x 25000 litre (m^3) recirculation raceway system.

Dietary Treatments

L-arginine, L-alanine, L-lysine and L-methionine at a concentration of 10^{-4}M were combined with Betaine at a concentration of 10^{-4}M. These will be presented to the fish in a dough ball form, consisting of a combination of maize/sorghum at (75/25%), there will be 5 treatments x 3 replicates in the experiment. Chemo-attraction as measured by the total fish count in the relative compartments after 10 and 30 minute intervals.

Free amino acids 10^{-4}M concentration

L- Alanine (batch no. 031104 Warren chem. Specialities) Mr = 89.09 n=0.0001 M=nxMr M=0.0001x89.09 = 0.008909ppt, therefore 8.909ppm.

L-Arginine (batch no. 04010389 Warren chem. specialities) Mr = 174.2
n =0.0001 M=0.01742ppt, therefore 17.42ppm.

L-Lysine (batch no. 030803 Warren chem. Specialities) Mr = 146.2
n = 0.0001 M=0.01462ppt, therefore 14.42ppm.

L-Methionine (batch no. 040702 Warren chem. Specialities) Mr = 149.2
n = 0.01 M=0.01492ppt, therefore 14.92ppm

Betaine hydrochloride 10^{-4}M concentration

Betaine hydrochloride (Batch number 2004050658, Warren Chem Specialities) Mr=156.6 n=0.0001 M=nxMr M=0.0001x156.6 = 0.01566ppt, therefore 15.66ppm.

Statistical analysis

Results for differences in chemo-attraction were analysed for significant differences using one-way ANOVA and Tukey's pair wise comparison test.

Results

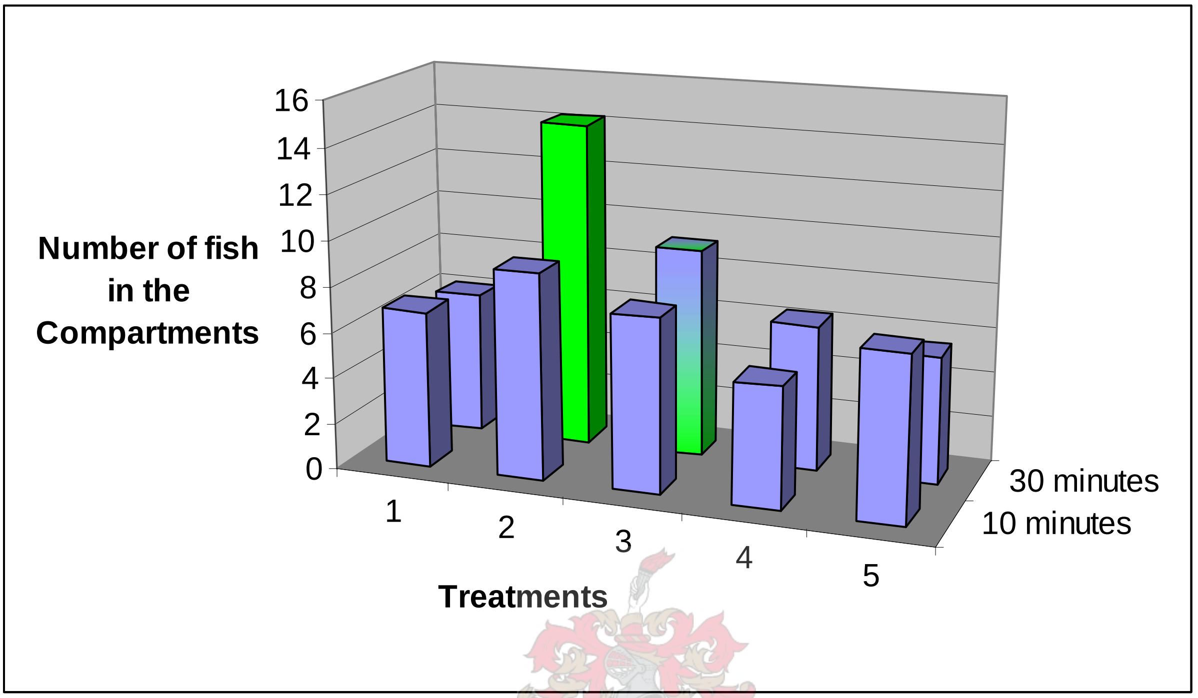

Figure 1. Influence of amino acids and betaine as an additive effect for Japanese Koi Carp (*Cyprinus carpio*)

Table 1. Means and standard errors for the additive effect of betaine hydrochloride with amino acids on chemo attraction in Japanese Koi Carp *(Cyprinus carpio)*.

Treatment	10 minutes	30 minutes
1. Betaine + Arginine	6.85± 1.51	6.21 ± 1.75
2. Betaine + Alanine	9.06 ± 1.59	14.3 ± 1.84[a]
3. Betaine + Lysine	7.67 ± 1.56	9.18 ± 1.80
4. Betaine + Methionine	5.33 ± 1.54	6.37 ± 1.78
5. Control	7.26 ± 1.46	5.58 ± 1.70

After ten minutes of the experiment, there was no significant difference (P>0.05) between the treatments. After thirty minutes of the experiment, there is a significant difference (P ≤0.05) in favour of treatment 2 (alanine + betaine) to that of treatment 1 (arginine + betaine), 4 (methionine + betaine) and 5 (betaine, control). There was no significant difference (P>0.05) between treatment 2 (alanine + betaine) and treatment 3 (lysine+ betaine), however there was a trend towards treatment 2 (alanine + betaine), mean value of 14.3.

Discussion

Individual amino acids and betaine are the most effective feeding stimulants for various crustaceans (Carr 1979; Carr 1978; Fuzessery et al. 1975; Heinen, 1980; Derby 1982). Responses of the receptor sites to amino acids: alanine or glycine may be enhanced by the presence of betaine in carp (Mayers, 1995).

Glycine and betaine especially have been identified as feed stimulants for a number of fish species, including Chinook salmon, Atlantic salmon and cod (Mayers, 1995). The palatability of alanine or glycine for red sea bream increased after adding taste-indifferent betaine into the diet (Goh & Tamura 1980). In extracts of blue crab and flathead grey mullet, a solution containing either betaine alone or the amino acids alone was only 2-9% as effective as the extracts themselves for pigfish and pinfish. Results from this trial indicate that alanine in combination with betaine activity as a stimulant is enhanced.

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Chapter 10

Conclusion

In conclusion to this study, a combination of cooked maize/sorghum 75/25% showed to be the best treatment for Japanese Koi Carp *(Cyprinus carpio)*, this conclusion was derived by statistical difference ($p \leq 0.05$) and trends in the mean values, between five cereals, cooked/uncooked maize and maize/sorghum combinations for the test on palatability.

In testing for chemo attraction in Japanese Koi Carp *(Cyprinus carpio)*, betaine hydrochloride at a concentrations of 10^{-4} (15.66ppm) showed the most satisfactory results ($p \leq 0.05$) as a feed stimulant. L-alanine is the best gustatory stimulant and L-lysine the olfactory attractant ($p \leq 0.05$). Mixtures of attractants are more effective than individual components. L-alanine in combination with betaine hydrochloride activity as a stimulant is enhanced, this can possible be explained due to the synergetic effect that betaine hydrochloride has on alanine.

In aquaculture supplementation of artificial, dry diets with attractants (feeding stimulants) can increase acceptability, and consequently the consumption of low palatability diets by fry and fingerlings. This practice can also reduce feeding time and feed waste, while improving water quality and environmental safety. Chemo-attractants are species specific and thus species have their own range of chemo-attractants. There is room for further research within the range of species related amino acids as chemo attractants.