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ASSESSMENT OF CHEMORECEPTIVITY IN GOLDFISH (*Carassius auratus*) FINGERLINGS TO IDENTIFY NATURAL FOOD ATTRACTANTS FOR FEED FORMULATION

CRYSTAL LIM LI YING

ASSESSMENT OF CHEMORECEPTIVITY IN GOLDFISH (*Carassius auratus*) FINGERLINGS TO IDENTIFY NATURAL FOOD ATTRACTANTS FOR FEED FORMULATION

CRYSTAL LIM LI YING

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It is hearby certified that we have read this project paper entitled "Assessment of Chemoreceptivity in Goldfish (*Carassius Auratus*) Fingerlings to Identify Natural Food Attractants for Feed Formulation", by Crystal Lim Li Ying and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Project.

ASSOC. PROF. DR. HASSAN HJ MOHD DAUD

B.Sc (UPM), M.Sc (SCOTLAND), Ph.D (ENGLAND)

Senior Lecturer,

Department of Veterinary Clinical Sciences

Faculty of Veterinary Medicine, Universiti Putra Malaysia

(Supervisor)

DR. HAFANDI AHMAD

B.Sc (UPM), M.Sc (UPM), Ph.D (LA TROBE)

Senior Lecturer,

Department of Veterinary Pre-Clinical Sciences

Faculty of Veterinary Medicine, Universiti Putra Malaysia

(Co-Supervisor)

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ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD 4999 Project.

ASSESSMENT OF CHEMORECEPTIVITY IN GOLDFISH (*Carassius auratus*) FINGERLINGS TO IDENTIFY NATURAL FOOD ATTRACTANTS FOR FEED FORMULATION

By

Crystal Lim Li Ying

2018

Supervisor: Assoc. Prof. Dr. Hassan Hj Mohd Daud

Co-Supervisor: Dr. Hafandi Ahmad

Goldfish (*Carassius auratus*) is one of the popular choice of fish hobbyist among the ornamental fish and usually kept in large numbers in aquarium or ponds. Thus type of feed and feeding routine are crucial to prevent overfeeding that will lead to the pollution of the aquarium. The assessment of chemoreceptivity in goldfish fingerlings was conducted to determine the chemoreceptivity between commercial fish pellet and the live feed. The aim was to suggest a formulation for the best diet using live food supplementation as feed attractants added to commercial pellet. In this experiment, the feeding behaviour of the goldfish were recorded using

GoPro recorder for 15 minutes after feeding them with six different pairs of feed formulation. Findings showed that the average time spent on bloodworm was the longest, followed by brine shrimp and mosquito larvae, while commercial pellet was the shortest. In addition, the highest frequency of number of entries preferred were for bloodworm, brine shrimp, mosquito larvae and commercial pellet accordingly. As a conclusion, the current study has indicated highly significant results from the time spent (P=0.001) and the number of entries (P=0.000) in each compartments, thus the goldfish was more receptive to the live fresh feed as compared to the commercial fish pellets as hypothesised.

Keywords: chemoreceptivity, live feed, commercial feed, time spent, *Carassius auratus*

ABSTRAK

Abstrak daipada kertas projek yang dikemukanan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada kerperluan kursus VPD 4999 Projek.

PENILAIAN KEMORESEPTIVITI DALAM IKAN EMAS (*Carassius auratus*) JUNENIL DALAM MENGENAL PASTI BAHAN PENARIK SEMULAJADI UNTUK FORMULASI MAKANAN

By

Crystal Lim Li Ying

2018

Penyelia: Prof. Madya Dr. Hassan Hj Mohd Daud

Penyelia bersama: Dr. Hafandi Ahmad

Ikan emas (*Carassius auratus*) adalah salah satu pilihan ikan yang popular di kalangan penggemar ikan hiasan dan biasanya dipelihara dalam jumlah yang besar di dalam akuarium ataupun kolam. Oleh itu, jenis makanan dan rutin makan adalah penting untuk mengelakkan pembaziran yang akan mengakibatkan pencemaran akuarium. Penilaian kemoreseptiviti di antara ikan emas juvenile telah dijalankan untuk menentukan kemoreceptiviti antara pelet ikan komersial dan makanan hidup. Tujuan eksperimen ini adalah untuk mencadangkan perumusan diet terbaik menggunakan suplemen makanan hidup secara langsung sebagai penarik makanan

yang ditambahkan kepada pelet komersial. Dalam eksperimen ini, tingkah laku pemakanan ikan emas telah direkodkan menggunakan perakam GoPro selama 15 minit selepas memberi makan kepada mereka dengan enam pasang penyediaan makanan. Dapatan menunjukkan bahawa masa purata yang paling panjang digunakan untuk mengidap adalah cacing darah, diikuti oleh artemia dan jejentik nyamuk, manakala pelet komersial adalah yang paling pendek. Di samping itu, frekuensi tertinggi bilangan penyertaan yang disukai oleh juvenil adalah cacing darah, artemia, jejentik nyamuk dan pelet komersial. Kesimpulannya, kajian ini telah Berjaya menunjukkan bahawa makanan semulajadi mempunyai kesedapan yang lebih tinggi berbanding dengan makanan komersial kerana ia mempunyai kemoatraktan yang lebih tinggi (P=0.001).

Kata kunci: kemoreceptiviti, makanan hidup, makanan pellet komersial, masa, *Carassius auratus*

1.0 INTRODUCTION

Fish keeping is an activity that colourful fishes were kept in an aquarium or garden pond at home. This hobby has live since thousands years ago, this can be supported by the evidence that the ancient Romans maintained elaborate ornamental marine fish ponds back in second century (Higginbotham, 1997). Ornamental fish come in a great variety of colour, species, shape and swimming pattern that gives people a tranquil and calming effect (Ng, 2016). In Malaysia, this industry started since the 1950's through collection of fish from natural water. Currently there is more than 250 species of 550 varieties of ornamental fish are cultured in Malaysia including the local species and exotic species from South America and Africa (Department of Fisheries, 2015). Goldfish (*Carassius auratus*) is the choice of the fish hobbyist among the ornamental fish due to the low maintenance and relatively cheaper price compare to other ornamental fish.

Owner tend to feed their goldfish with commercial pellet only which lacks of variety of other feed indirectly lead to the problem of underfeeding. Underfed goldfish tend to produce abundant of leftover food which lead to the pollution of the fish tank and deterioration of the water quality. Low water quality causing stress and immunosuppression to the fish leading to the decline of the fish health. Therefor a good aquarium management and the formulation of specific feed for the variety of the ornamental fish is crucial to prevent this problem from happening (Ostrow, 2003).

With the concern stated above has led to my project regarding the assessment of chemoreceptivity in Goldfish (*Carassius auratus*) juvenile to identify the preferred food attractants for the feed formulation especially for goldfish. Feed selection from a fish is decided by the animo acid content in the feed through chemoreception. Chemoreception defines as the physiological response of a sense organ to a chemical stimulus which through olfactory and gustation (Green & Zielinski, 2014). In this project, goldfish juveniles were used as they are more naïve in selecting the most attractive feed through their olfactory sense. The choices of feed for goldfish are commercial fish pellets, bloodworm, mosquito larvae and brine shrimp.

Realising with the issues stated, this study was undertaken with the objectives to determine the chemoreceptivity between commercial fish pellets and fresh feed for goldfish juvenile. And with that to suggest a formulation of the best diet for goldfish juveniles using live food supplementation as feed attractants. The expected outcome would be goldfish is receptive to the live fresh feed compared to the commercial fish pellets.

2.0 LITERATURE REVIEW

2.1 Goldfish chemoreceptivity

Goldfish (*Carassius auratus auratus*) was bred in China, they are the offspring of Prussian Carp (*Carassius auratus gibelio*) which originated in Central Asia (Linnaeus, 1758). But, carp have visible barbels near their mouths but missing from goldfish. A common goldfish is one of the flat-bodied goldfish. The fin of a common goldfish is held fully erect with the caudal fin moderately forked. The dorsal fin begins at the highest part of the fish's back and extends along the back (Ostrow, 2003).

Chemoreception defines as the physiological response of a sense organ to a chemical stimulus (Green & Zielinski, 2014). Fish are immersed in their chemical and physical environment, and their sensory systems provide them information for feeding, defence, and reproduction (Green et al., 2013). Chemoreception can be categorised into two main system which are olfactory and gustation. In goldfish, the olfactory organs are located in the nostril and it does not connect to the mouth or throat like other higher animals do. Goldfish has two nasal opening and each one is a dead sac (Ostrow, 2003). Water enters and leaves the nostril through the same opening. Meanwhile, gustatory system contribute to the final evaluation in the feeding process (Kasumyan *et. al.,* 2003) in capturing the feed.

The sensory cells responding to the chemical signals are exposed to the environment through communication to the brain where neuronal integration ensues, and the motor output is targeted to specific muscle groups and then leads to a

behavioural response. However, there is difference on how the chemical compounds can be disperse in an aquatic environment compared to the terrestrial environment. Firstly, the molecules have to be in liquid form rather than gaseous form to be distributed. Secondly, water is a slower carrier medium when compared to the air and have to be transported through diffusion (Atema, 1980). Hence, solubility determines the type of compounds that can be found and utilized as chemical signals.

2.2 Feeding habits

The right feeding of goldfish is another important criteria of good aquarium management.

Overfeeding tend to produce more faces than normal. If they overeat all the time, the wastes that produces by these goldfishes will be more than the filter system could handle. Even the weekly change of water cannot compensate for this much waste and the aquarium will be polluted sooner or later beyond the tolerance of the goldfish.

The amount of the feed should never be given more than the goldfishes can finish in 5 minutes including the time they spent picking food from the bottom of the tank. Goldfish juvenile should be fed two to three times a day but an adult goldfish should not be fed more than once a day. An overfed goldfish then to become fat, lethargy, and most unlikely to resist trauma and disease. The adult goldfish that being

fed once a day tend to remain active and healthy throughout many years beyond its sexual maturity (Ostrow, 2003).

2.3 Feed palatability

According to Glencross et al. (2007), feed palatability is defined as acceptable to the taste or agreeable in flavour to be eaten. Feed acceptance depends on a variety of chemical, nutritional and physical characteristics, all of this can be affected by the choice of feed ingredients and type of processing used in the feed manufacturing (Jobling et al., 2001). There are various quality of commercial pellet available in the market. High quality commercial pellet tend to have higher fibre content but with low palatable. Low quality of commercial pellet has higher carbohydrate content compare to others. The ability of the fish to ingest and the detect the feed can be caused by the properties of the feed such as pellet density, size, colour and texture (hardness) and the chemical composition of the feed which will depend in the ingredients used. Protein is the important ingredient that fingerlings need to eat.

2.4 Feed sample

2.4.1 Artemia cysts (Brine shrimp eggs)

Artemia are primeval aquatic crustaceans which can be found in saline lakes in tropical and subtropical country (Gajardo & Beardmore, 2012). The common used artemia is known as *Artemia salina* is also known as brine shrimp. It belongs to the phylum Arthropoda, class Branchiopoda and forms a member of the zooplankton community like copepods. The Artemia life cycle begins by the hatching of dormant cysts, can remain dormant for many years as long as they are kept dry, cool and protected from UV radiation (Stappen, 1996).

When the cysts are placed into salt water, they readily rehydrate and resume the encased embryos resume their interrupted metabolism. After about 20 hours, the outer membrane of the cyst bursts and the embryo can be seen. The embryo hangs underneath the empty shell which indicating the umbrella stage. The development of the nauplius is completed and within a short period the hatching membrane is ruptured, release the free-swimming nauplius (Anufriieva & Shadrin, 2014). The first larval stage which is also known as instar I has a brownish-orange colour, a red nauplius eye and three pairs of appendages. The instar I larva only rely on the yolk reserves and does not take up food as its digestive system has not fully developed yet. In this stage, their egg yolk will be very nutritious to feed the fish (Stottrup, 2008). They had higher protein and lipid compared to the other stage of the artemia. After about 8-12 hours, it developed into second larval stage also known as instar II nauplii. In this stage, the fat content reduce gradually. They reach adult in 8 days which rich in protein.

Plus, decapsulation of Artemia cysts supply numerous advantages, such as disinfection, improved hatchability, easy storage, and is less expensive and labour

intensive than regular hatching (Bruggeman et al., 1980). Even low-quality cysts can be used as a food source (Ribeiro and Jones, 1998), and decapsulated cysts preserve a higher energy content than freshly hatched nauplii (Vanhaecke et al., 1983).

2.4.2 Bloodworm

Worms have been used as fodder for fish due to the numerous protein content in it. Bloodworms are referred as one of the good fish-fodder whether they are fresh or frozen. Bloodworms belongs to the *Chironomidae* family, which are well known fresh water organisms due to their significance in aquaculture (Ashe & Cranston, 1990)They are known as bloodworm due to the red colour caused by the presence of haemoglobin in the body fluid. This allows them to take oxygen in low oxygen waters. The Family Chironomidae plays a significant role in aquatic food webs, representing a major link between producers, such as phytoplankton and benthic algae, and secondary consumers (Tokeshi 1995). They usually harvested from floodplain of the large rivers which is a flood area of the river runoff (Welcomme, 1983).

Bloodworm is a major natural food for various species of freshwater fish, including goldfish (Komatsu et al., 2000). Moreover, bloodworm contains the nutrients that suit the needs of the freshwater fish and favoured by fish (Gupta and Banerjee, 2009). Frozen bloodworms are easier to be digested compared to

earthworm. They are more productive and have excellent digestive coefficient in fish (De La Noue et al., 1985).

High protein of this worm makes them an excellent choice for sturgeon fish larvae as starter feed (De La Noue et al., 1985). Other than that, bloodworms are also the good resource of lipid, minerals and vitamins (Habib et al., 1997). With this, the worm could be used as dietary supplement. According to Sugden (1973), the bloodworm contain 56% protein and has about 73.6% digestibility. However, utilization of bloodworm as fish feed in aquaculture is still very limited due to the price of bloodworm is higher compared to fish pellet the bloodworm production depending on the harvesting from the nature.

2.4.3 Mosquito larvae

Mosquito larvae are the larvae stage of blood sucking mosquito. They can be found in most of the water media such as pond where fish live. The embryonic development of the mosquito usually are ready to hatch in 4 -8 days after oviposition, if the eggs are kept between 20-25ºC, reported by Becker (1989). It is the ideal natural non-polluting food for the most fishes in the pond. According to Underwood (1901), he notice that there is reduction of mosquito larvae in the pond ever since the goldfish was introduced into the pond. Goldfish fry will double their growth rate if they get enough of the mosquito larvae. Besides, it is known that mosquito larvae has high lipid content mainly saturated fatty acids.

2.4.4 Commercial fish pellet

Pelleting is the processing of small particle into larger particle by using mechanical process combined with heat, pressure and moisture (Falk, 1985). It changes the homogenous blend of dry ingredients into a form that make its physical characteristics suitable for feeding purpose. Drying is also done in the process of producing the dry pellet to remove the moisture in the feed. This process enhances the durability of the feeds for the proper storage. The preference of the feed intake by the fish decided by the properties of the feed such as pellet density, size, colour and chewiness and the chemical composition of the feed which depend on the raw material used to process the pellet. The size of the pellet used depends on the size of the fish to be fed. Fish pellet comes in two form which are extruded which the pellet will float on the water or pressure-pellet where the feed will sink to the bottom of the tanks depends on the type of feeder which the targeted fish is.

3.0 MATERIALS AND METHODS

3.1 Acclimatization of the fish

A species of goldfish fingerlings were used in this project, which is common goldfish (*Carassius auratus*) due to its hardiness which suitable to run an experiment. During the experiment, the size of fishes ranged around 5.5cm in length and weighing 2.5g. Ten common goldfish fingerlings were purchased from Sanwa Aquatic Sdn. Bhd., an ornamental fish wholesaler in Sungai Buloh. The fingerlings were transported to the aquatic laboratory in Faculty of Veterinary Medicine UPM and kept in an aerated aquarium tank seven days before the experiment for acclimatization of the fish. The fingerlings were fed once daily with commercial fish pellet 2% from its body weight and will be fasted one day before the experiments.

3.2 Preparation of feed solution

Four different ingredients were picked and processed into 4 testing solutions, such as:

- Commercial goldfish pellet
- Bloodworm (frozen)
- Mosquito larvae (self-prepared into frozen cube)
- Brine shrimp (Brand: Bio-marine)

The mosquito larvae were collected from a container with stagnant water and rinsed with distilled water before being centrifuged in 14,000 rpm for 10 minutes (Arunprasanna et al., 2017). And then it was transferred into ice cube tray added with distilled water enough to cover the larvae before freeze under 0^{o}C. All the frozen

feed is thawed in room temperature before used to retain the freshness and palatability.

All of the feeds stated above were prepared into a 1% concentration feed solutions since it was found had the fastest response (Yeap, 2016). Approximately 1.5g of each feed stated above was crushed into smaller pieces using pestle and mortar, and then diluted with 148.5ml of aged tap water to get a 150ml of 1% concentration of feed solution. The other feed also prepared by using the same method. The remaining solution were discarded after use and the new dilutions were prepared with the same manner to prevent spoiling that might affect the result.

3.3 Preparation of the three-compartment maze

A fish tank was modified into a three-compartment maze and it was place on the table 90cm above the ground and the all of the surface of the maze was wrapped with black plastic paper. The tank is divided into three compartments with 2 gates made from hard cardboard. The tank was filled with aged tap water and the water temperature maintained at room temperature which is at 25ºC. The lighting and volume of water in the maze were kept the same throughout the experiment. Please refer to Appendix 1 for the setup of the three-compartment maze.

3.4 Experimental work-flow

The experimental was divided into two parts, which were the feed solution response test and the feed choice test. Response test was done to identify to see whether the fish will be attracted to the feed located in the maze. After identifying the feeds that the fish was attracted to, the feed choice test was done to compare the attractiveness between the selected feed for goldfish juveniles.

3.4.1 Feed Solution Response Test

Steps in running the test:

i. The 3-compartments maze was filled up with deionised distilled water with to a depth of 9 cm. Both gates will be blocked at each compartment before the experiment begins.

ii. Two goldfish fingerlings were placed in the middle compartment and allowed to acclimatize for 20 minutes (Ostrow, 2003).

iii. Firstly, 5 ml of 1% commercial fish pellet solution was introduced into L compartment.

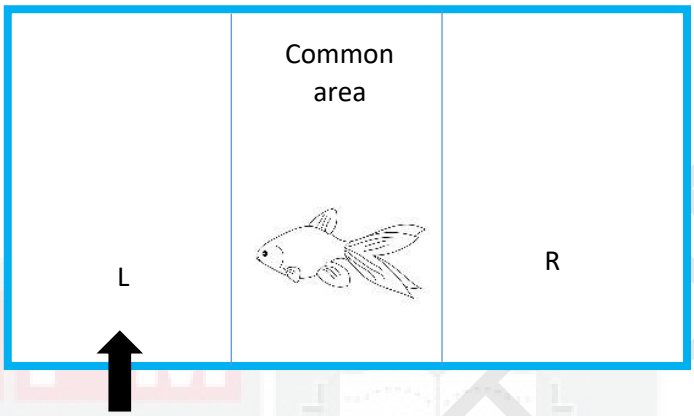

Figure 1.0: Diagram showing the lay-out of the compartments where feed solutions were placed

i. The timer was started when both gates were lifted simultaneously to expose the L and R compartments to the fish. The gates were lifted carefully to minimize the disturbance occurred to the fingerling.

ii. The behaviour of the fingerlings were observed through installed action camera and the directions where the fingerling headed were recorded continuously for 15 minutes.

iii. After the recording the fingerlings were removed and the compartment was cleaned.

iv. Step i. to vii. were repeated with the following solutions at 1% concentration of :

   a. Bloodworm

   b. Mosquito larvae

   c. Brine shrimp

3.4.2 Feed choice test

Steps in running the test:

i. The 3-compartments maze was filled up with deionised distilled water with to a depth of 9 cm. Both gates will be blocked at each compartment before the experiment begins.

i. A pair of goldfish fingerlings were placed in the middle compartment facing the other two compartment (L & R) and allowed to acclimatize for 20 minutes (Ostrow, 2003).

ii. Finally, 5 ml of 1% commercial fish pellet solution and 5ml of bloodworm solution were introduced into L & R compartments simultaneously at the same time. This step was done 5 minutes before the removal of the gates.

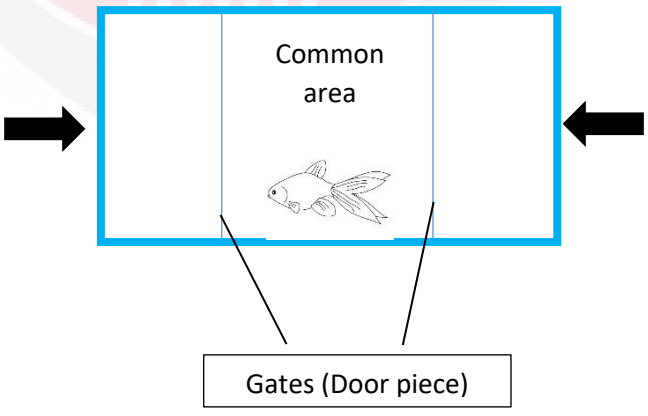

Figure 1.1: Diagram showing the lay-out of the compartments where feed solutions were placed

iii. The gates were carefully removed at the same time to expose the L and R compartments to the fish and to minimize the disturbance done to the fingerling. The timer was then started.

iv. The behaviour of the fingerlings were observed through installed action camera and the direction where the fingerlings headed was recorded for 15 minutes.

v. Finally, the fingerlings were removed and the compartment was cleaned.

vi. Step i. to vii. were repeated with the following pairing of feed solutions:

Table 1.0: Feed pairing

FF 1	Commercial pellet vs bloodworm
FF 2	Commercial pellet vs mosquito larvae
FF 3	Commercial pellet vs brine shrimp
FF 4	Bloodworm vs mosquito larvae
FF 5	Bloodworm vs brine shrimp
FF 6	Mosquito larvae vs brine shrimp

vii. Each feed pairing (Step i. to viii.) was repeated three times with different goldfish fingerlings.

4.0 RESULTS

4.1 Response Test

The fingerlings showed positive behavioural changes towards the introduction of feed solutions (Table 4.1.1). The fingerlings swam to the compartments where the 1% of feed solution was placed. The fingerlings were then tested on commercial pellet, bloodworm, mosquito larvae, and brine shrimp with the same concentration of 1% solutions. However, none of the fingerlings responded to the negative control where there is nothing placed in opposite compartment.

Table 4.1: Response Test

	Choice between feed	
	Empty (Negative control)	Commercial pellet
Fingerling 1		✓
	Empty (Negative control)	Bloodworm
Fingerling 2		✓
	Empty (Negative control)	Mosquito larvae
Fingerling 3		✓
	Empty (Negative control)	Brine shrimp
Fingerling 4		✓

4.2 Feed Choice Test

Results shown in Figure 4.2.1 to 4.2.3 indicated that the goldfish fingerlings preferred fresh feeds such as bloodworm, mosquito larvae and brine shrimp over the commercial pellet with the significant P-value of 0.002, 0.000 and 0.009 respectively. The data was interpreted by paired T-test since 2 types of feed were compared at a time. However, when compared among the fresh feeds (Figure 4.2.4 to 4.2.6), there were significant differences between bloodworm and brine shrimp

(P=0.001, P<0.05). But there was no significant difference between bloodworm and mosquito larvae (P=0.845, P>0.05), mosquito larvae and brine shrimp (P=0.829, P>0.05).

One way analysis of variance (ANOVA) test was done to compare the preferred feed among the four types of feed tested. Table 4.2.7 showed the time spent on bloodworm (8.00±2.60) was the longest among the feed, followed by brine shrimp (6.87±2.58), then mosquito larvae (6.80±3.27), lastly commercial pellet (4.97±3.34) which had the least mean value. A bar chart was plotted as shown by Figure 4.2.7.

Figure 4.2.1: Mean time spent between commercial pellet and bloodworm

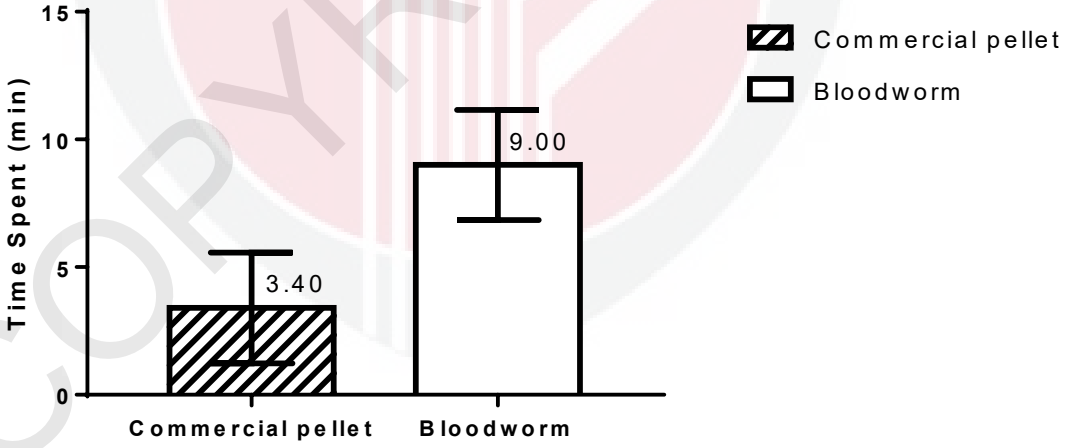

Table 4.2.1: Paired T-test for commercial pellet and bloodworm

	N	Mean	SD	SEM	95% CI for difference	P-value
Commercial pellet	10	3.90	2.60	0.82	(-7.28, -1.92)	0.002
Bloodworm	10	8.50	3.06	0.97		

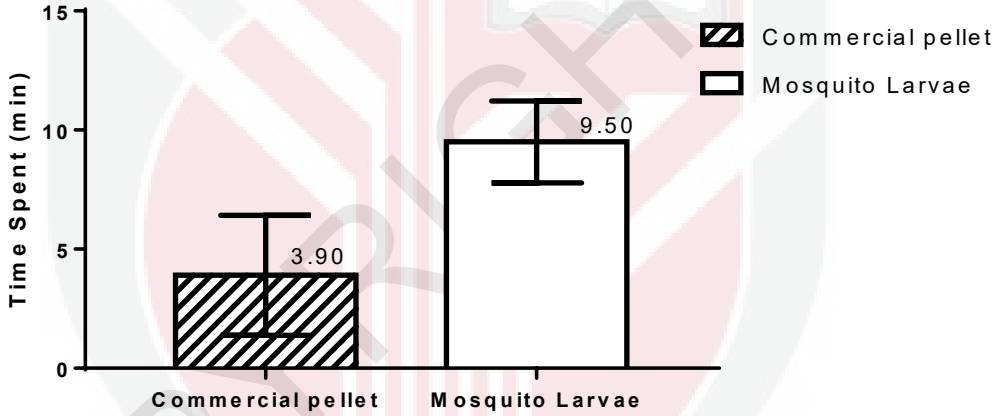

Figure 4.2.2: Mean time spent between commercial pellet and mosquito larvae

Table 4.2.2: Paired T-test for commercial pellet and mosquito larvae

	N	Mean	SD	SEM	95% CI for difference	P-value
Commercial pellet	10	3.90	2.51	0/80	(-7.652, -	0.000
Mosquito larvae	10	9.50	1.72	0.54	3.548)	

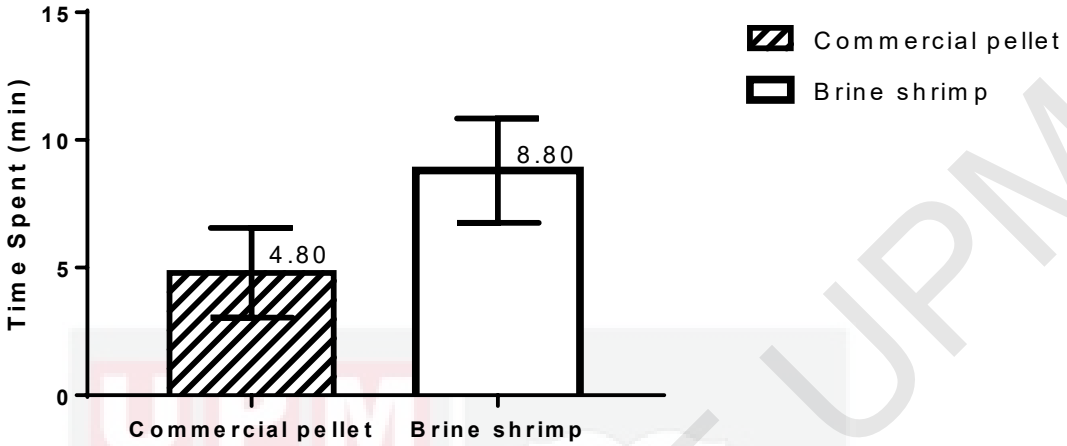

Figure 4.2.3: Mean time spent between commercial pellet and brine shrimp

Table 4.2.3: Paired T-test for commercial pellet and brine shrimp

	N	Mean	SD	SEM	95% CI for difference	P-value
Commercial pellet	10	5.40	2.07	0.65	(-4.814, -0.786)	0.009
Brine shrimp	10	8.20	2.20	0.70		

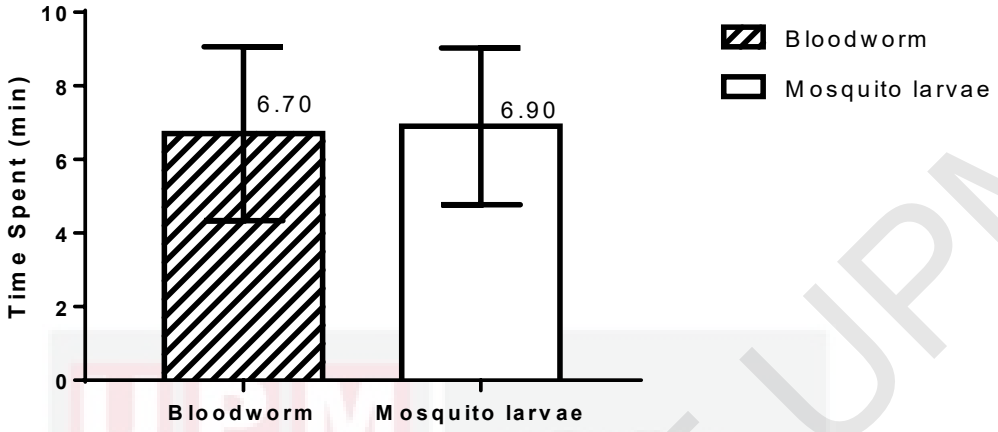

Figure 4.2.4: Mean time spent between bloodworm and mosquito larvae

Table 4.2.4: Paired T-test for brine shrimp and mosquito larvae

	N	Mean	SD	SEM	95% CI for difference	P-value
Bloodworm	10	6.70	2.36	0.75	(-2.32, 1.92)	0.845
Mosquito larvae	10	6.90	2.13	0.67		

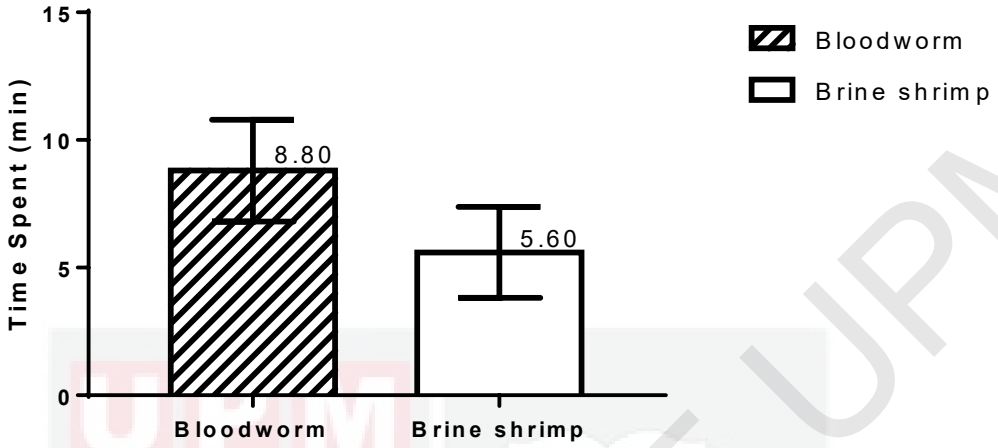

Figure 4.2.5: Mean time spent between bloodworm and brine shrimp

Table 4.2.5: Paired T-test for bloodworm and brine shrimp

	N	Mean	SD	SEM	95% CI for difference	P-value
Bloodworm	10	8.80	1.99	0.63	(1.421, 4.979)	0.001
Brine shrimp	10	5.60	1.78	0.56		

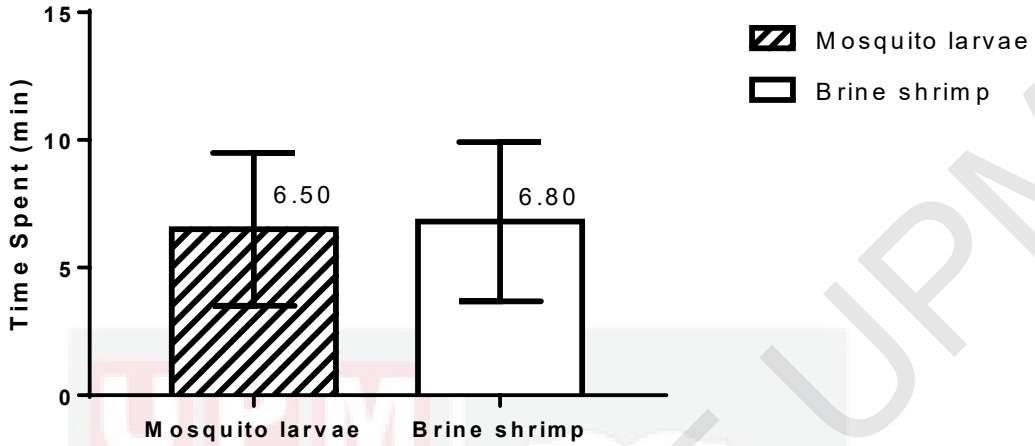

Figure 4.2.6: Mean time spent between mosquito larvae and brine shrimp

Table 4.2.6: Paired T-test for mosquito larvae and brine shrimp

	N	Mean	SD	SEM	95% CI for difference	P-value
Mosquito larvae	10	6.50	2.99	0.95	(-3.18,	0.829
Brine shrimp	10	6.80	3.12	0.99	2.58)	

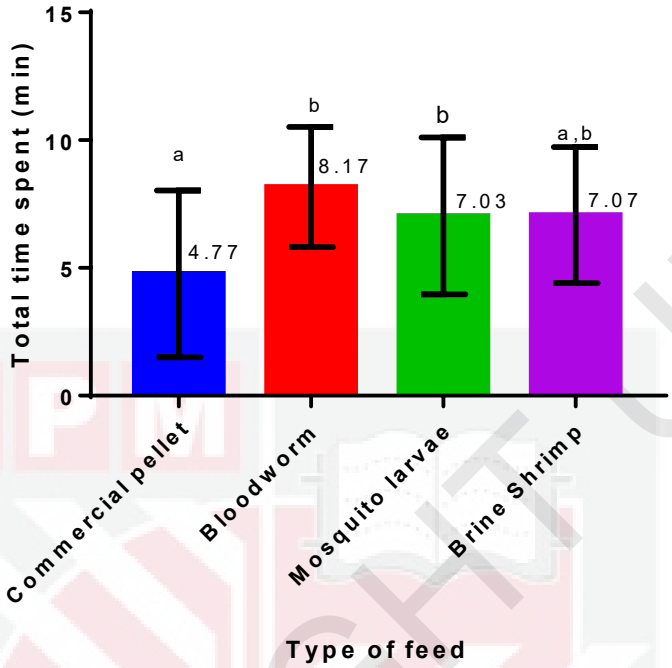

Figure 4.2.7: Total mean time spent among the 4 types of feeds

Tukey test was done on the number of entries into the compartment with the selected four different type of feeds. The test showed that the only pair that has significant difference (Figure 4.2.9) is commercial pellet and mosquito larvae (P=0.034). Otherwise, the rest of the feed pair showed insignificant difference between results (Figure 4.2.10-13). One-way analysis of variance (ANOVA) test was done to compare the number of entries to the compartments among the four feeds. The graph (Figure 4.2.14) indicated that bloodworm (6.47±3.40) has the highest frequency among the feed, followed by brine shrimp (6.00±2.58), then mosquito larvae (4.90±2.59), and lastly commercial pellet (2.97±2.34) which showed the lowest frequency.

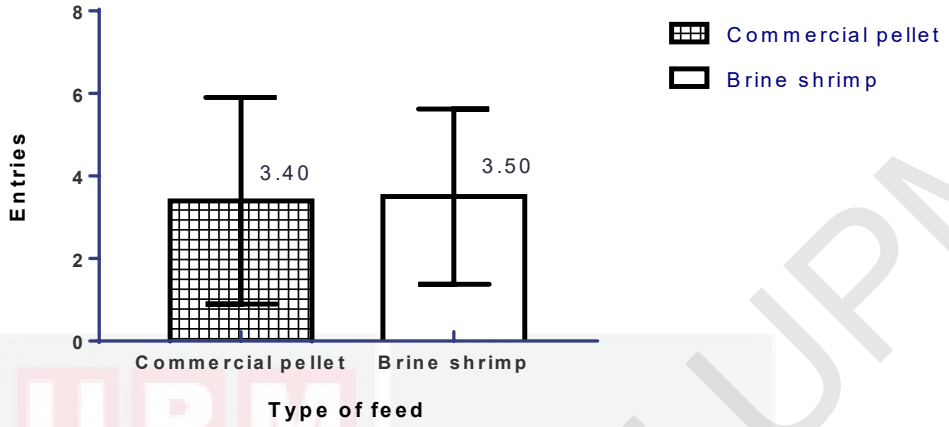

Figure 4.2.8: Mean number of entries between commercial pellet and bloodworm

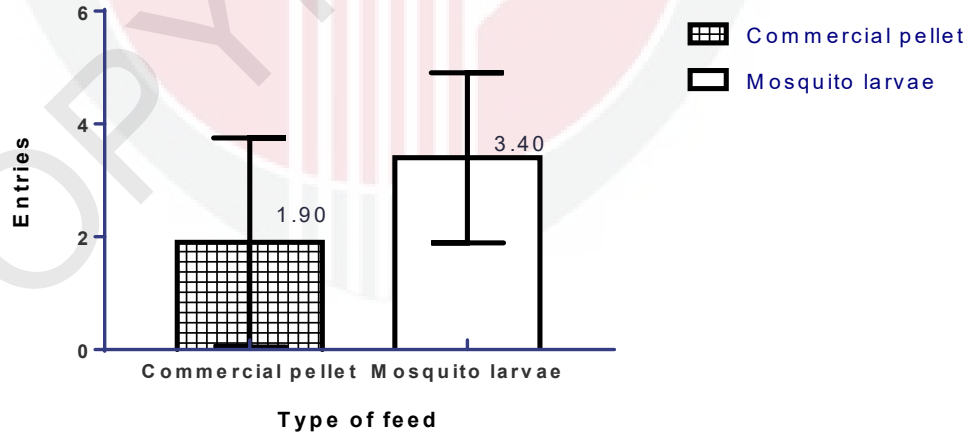

Figure 4.2.9: Mean number of entries between commercial pellet and mosquito larvae

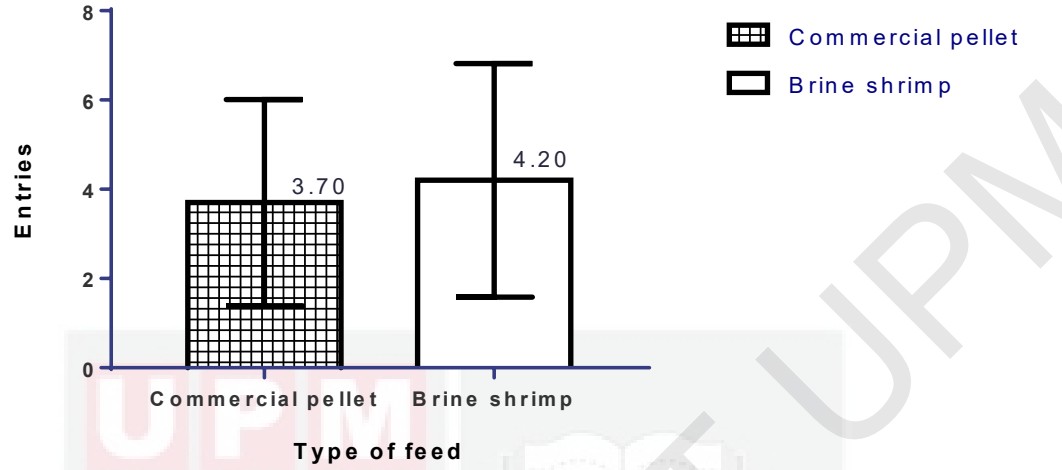

Figure 4.2.10: Mean number of entries between commercial pellet and brine shrimp

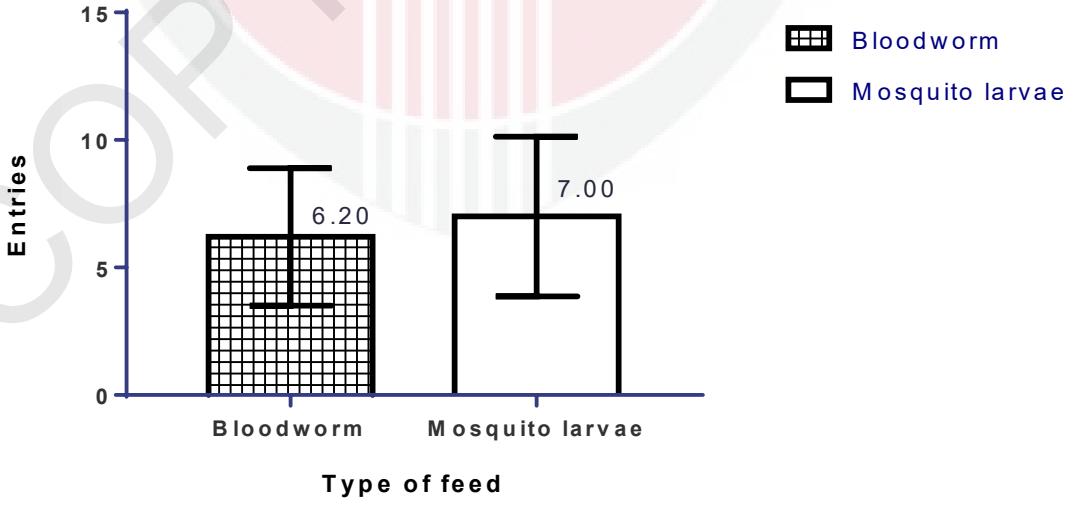

Figure 4.2.11: Mean number of entries between bloodworm and mosquito larvae

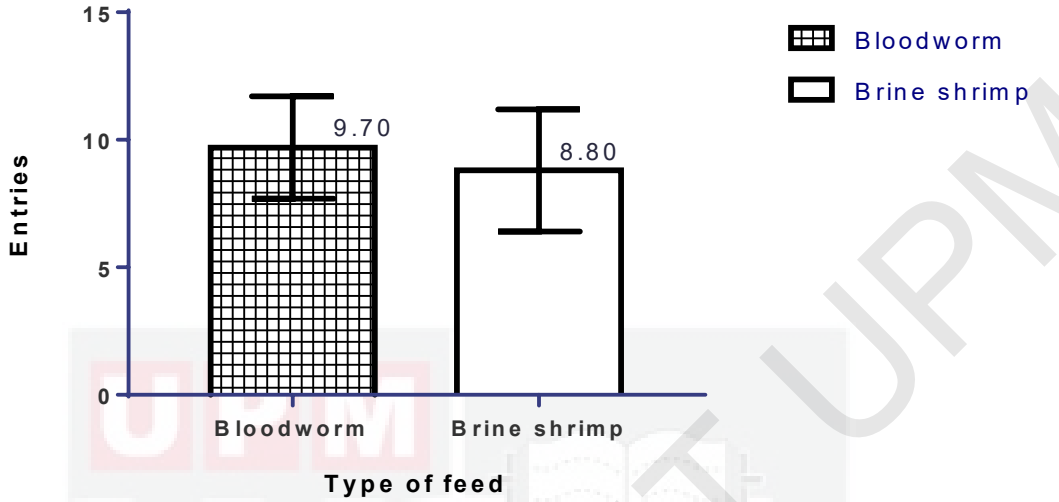

Figure 4.2.12: Mean number of entries between bloodworm and brine shrimp

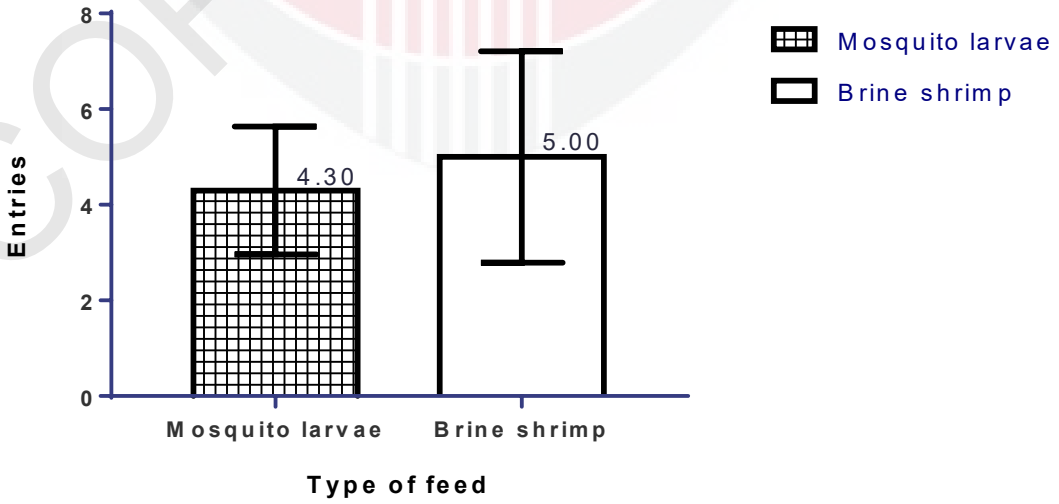

Figure 4.2.13: Mean number of entries between mosquito larvae and brine shrimp

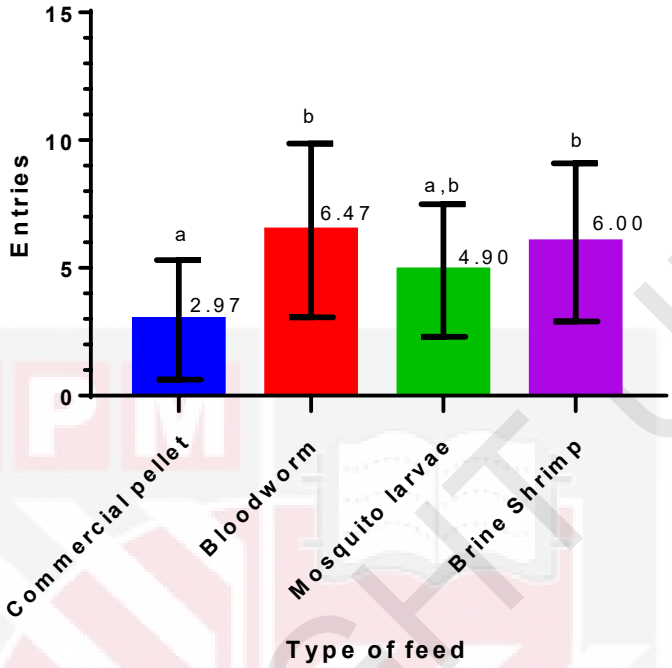

Figure 4.2.14: Number of entries recorded among the four types of feeds

5.0 DISCUSSION

Fishes receive the information regarding food through a sensory system called chemoreception that could be grouped into two main sensory systems which are olfactory organ and gustation organ (Green *et. al.,* 2013). In goldfish, the olfactory sensory neurons consist of cilia which express receptor genes resembling those that characterize the mammalian olfactory epithelium which aid in feeding stimulated by amino acids (Eisthen, 2004). On the other hand, gustatory organ which is the taste buds, are distributed on gill arches and rakers , and also situated on the appendages as well as the oropharyngeal cavity (Hara, 1994). This enables goldfish to have a remarkable sensory sense.

In the response test, all the test subjects which were the goldfish fingerlings, showed a positive feedback towards the feed. When they perceived the presence of food in the nearby compartment, they started to swim towards the said compartment. This behaviour could be distinguished from regular swimming due to the change in swimming patterns. Before the food was placed, the goldfish was swimming calmly at the bottom of the tank. Once the barriers between compartments were lifted, there was an obvious change in swimming pattern towards the food. This condition was supported by a report from Ronnestad (2013) stating that feeding behaviour in fish comprises of few activities such as detection, capture and ingestion that happens before and after the consumption of food. According to Kasumyan *et. al.,* (2003), when a fish with well-developed olfactory system senses the food, the fish will initiate searching by making a circle or S-shaped loops to reach the object which was

similar to observations observed in the current study. This was categorised as extraoral taste response.

In the feed choice test, there were two parameters being studied which were the total time spent in each compartments and the number of entries into the compartments. In the current study, the goldfish fingerling spent the most time (mean) in the food compartment containing bloodworms. This was followed by brine shrimp and mosquito larvae. The goldfish fingerling spent the least time (mean) in the food compartment containing commercial pellets. According to Hepher (1988), feed intake was affected by few factors such as food composition, fish size and the feeding habits. The composition of each feed was different which affected the preference of the goldfish fingerlings. Based on the calculated result, there was a significant difference in P-value among the commercial pellet and the other three fresh feed, meaning that the goldfish fingerlings actually preferred the other three feed i.e. bloodworms, brine shrimp and mosquito larvae as compared to commercial pellet. This findings were in agreement with Chikaraishi et al. (2015) whom stated that the commercial pellet composed of approximately 30% of protein, 5% of carbohydrate, 4% of lipids and 12% of lime, while bloodworms contribute large portion of protein and lipid in the diet. According to Czeczuga et al. (1973) bloodworm consists higher amount of aspartic acid, glutamin acid and alanine which composed of 10.4%, 8.5% and 7.7% from the protein respectively. This means the goldfish is attracted to alanine, glutamin acid and aspartic acid. In addition to that, there is another reason why goldfish is more attracted towards bloodworms as bloodworms exist when carps, the ancestor of goldfish, presented about 200 million

years ago. Bloodworm were a dominant life form in the freshwater habitats as one of the readily available food sources where the goldfish ancestors evolved (Hopkins, 2007). However, there's no significant result on comparing between the freshly prepared feed.

In regard to the second parameter, which is the number of entries into the different feed compartments, Tukey test was done to calculate the significance. From the analysis, there is no significant difference in almost all the feed formulation pair. However, the insignificant value can be explained that the behaviour of the goldfish fingerlings tend to stay longer in the preferred compartments, spending time to eat the fresh feed explaining the low number of entries. Dhert *et. al.,* (1997) found that juvenile of grass carp, common carp, and barbel carp grew better on brine shrimp compared to commercial starter feed because of the higher protein content of brine shrimp. This statement can be supported by the study done by Conceic *et al,* (2004) stated that abundant of alanine found in the yolk-sac of the brine shrimp which attracts the fish. In my study, the brine shrimps were introduce 2 days after hatching which the yolk-sac still remain intact. The third place goes to mosquito larvae which supported by the study done by Gupta stated that goldfish can be popularised as an efficient mosquito biological control agent. When comparing the three fresh feed, there are no significant difference between time spent in the compartments containing mosquito larvae with brine shrimp and mosquito larvae with bloodworm. The results can be interpreted as the goldfish fingerling showing almost equal receptivity to mosquito larvae, bloodworm and brine shrimp due to the freshness and palatability which lead to the almost same frequency of entries between the fresh

feed. The motive of the one way analysis of variance (ANOVA) test has been carried out to compare the preference of feed among the 4 types of feed.

The ornamental fish industry can be improved by identifying feeds with good palatability and adding them to commercial feeds as feed attractants. These food attractants can be used to replace a diet ingredients depending on the form of feed and the fishes' nutritional requirements. This allow the improvement of a feed's palatability causing less feed wastage. Feed with good palatability encourage the consumption of the feed leading to a better feed conversion ratio which the feed cost can be reduced. More importantly, less feed wastage could reduce the tank's pollution and deterioration of the water quality which would lead to the decline of fishes' health.

The limitation of this experiment was run one goldfish at a time in the three compartment maze. The goldfish seems nervous and tend to be static for the rest of the half an hour which show no response to the feed. One more goldfish was added into the tank. This had a positive effect as the goldfish fingerlings showed good response towards the feed with less sign of nervousness and stressed. In the end, a consistent result was obtained from the set up of two goldfish in 3-compartments maze.. In addition, another challenge was to get enough amount of mosquito larvae of almost the same size or stage within a limited time. The rearing of mosquito larvae started a week prior to the experiment however it was insufficient and the experiment was forced to be delayed for few days as this feed is not commercialised produced and had to be obtained from the environment.

Finally, findings of the experiment concluded that the alternative hypothesis was accepted whereby the goldfish was more receptive to fresh feed as compared to the commercial fish pellets following the paired T-test analysis carried out.

6.0 CONCLUSIONS & RECOMMENDATION

The significant result of the current study showed that the goldfish fingerlings was more receptive to fresh feed as compared to the commercial fish pellet. Among the fresh feed tested, the goldfish fingerlings preferred bloodworm, mosquito larvae and brine shrimp accordingly. It is believed that the bloodworm has the highest and correct amino acids profile which enhanced its palatability and has chemo attractiveness to the fingerlings. It is known that every species of fish is attracted to different kind of amino acids. The outcome of the experiment could be used as the feed attractants to add to commercial pellet to increase the feeding stimuli, feed conversion ratio (FCR) and reduce the feed wastage.

For the future research, it is suggested that similar experiment can be carried out with different species of fish and a different variety of live feed, either insects or algae. It's also recommended to analyse the nutrition content of each feed to identify the type of amino acids that attract the fish. The set-up of the maze can also be improved to test more than two feeds simultaneously.

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APPENDIX 1: THREE-COMPARTMENT MAZE SETUP

A fish tank covered with black plastic will be used throughout the experiment. The measurement of the aquarium was 33cm x 20cm x 22cm in rectangular shape. Two hard black cardboards served as the separator to create a three compartments maze. The cardboards were covered with plastic that fit perfectly to the tank to make it waterproof. An action camera is place higher from the tank to get a top view with the aid of a tripod.

After setting up the maze, it was cleaned with aged tap water before the acclimatization of the goldfish fingerling and the experiment.

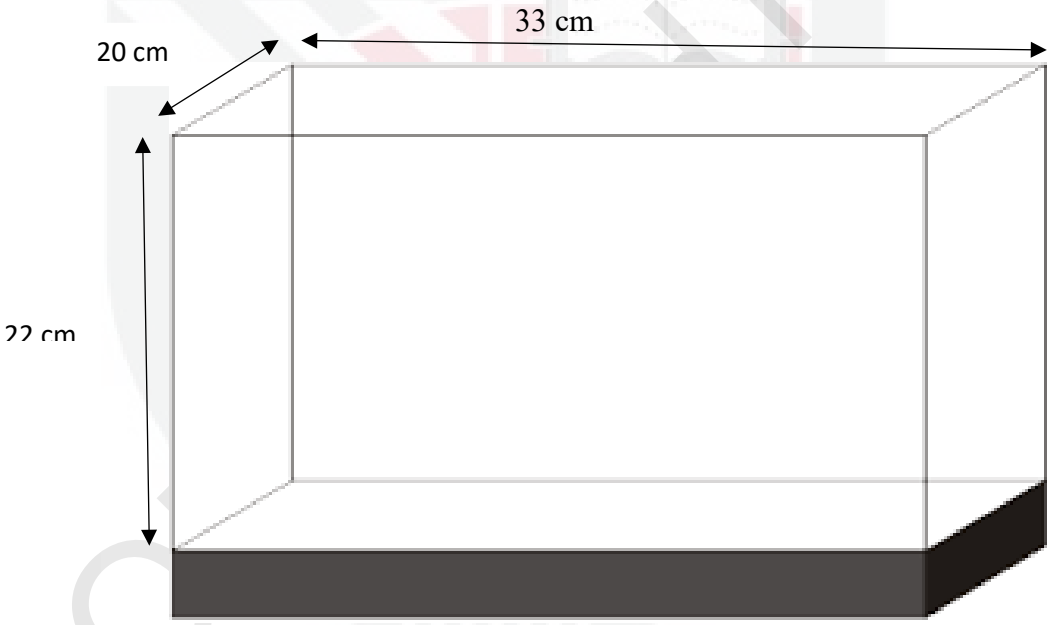

Figure 5.0: Dimension of the fish tank used as maze

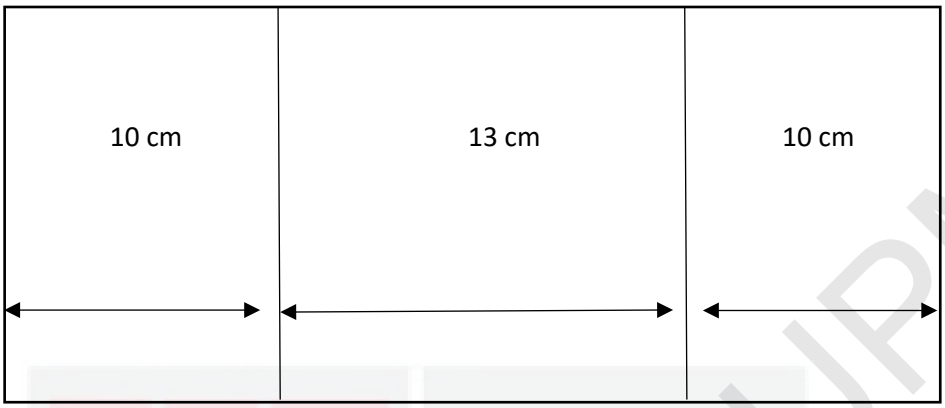

Figure 5.1: View of the tank from the top with the gate

APPENDIX 2: RESULT SHEET

Response Test

	Choice between feed	
	Distilled water (Negative control)	Commercial pellet
Fingerling 1		/
	Distilled water (Negative control)	Bloodworm
Fingerling 2		/
	Distilled water (Negative control)	Mosquito larvae
Fingerling 3		/
	Distilled water (Negative control)	Brine shrimp
Fingerling 4		/

Feed Choice Test

FEED FORMULATION 1

Replicate	Choice between 2 feeds			
	Commercial pellet		Bloodworm	
	Time (mins)	Entries	Time (mins)	Entries
1	8	4	2	2
2	6	3	8	4
3	5	8	8	8
4	3	6	12	3
5	5	4	7	2
6	6	5	6	2
7	1	1	11	4
8	0	0	10	3
9	4	1	9	1
10	1	2	12	6
Total	39	34	85	35

FEED FORMULATION 2

Replicate	Choice between 2 feeds			
	Commercial pellet		Mosquito larvae	
	Time (mins)	Entries	Time (mins)	Entries
1	0	0	10	4

2	0	0	10	4
3	4	5	10	3
4	3	3	10	5
5	12	1	3	3
6	0	0	15	3
7	11	1	2	1
8	10	1	5	1
9	9	2	5	5
10	7	5	7	5
Total	56	18	77	31

FEED FORMULATION 3

Replicate	Choice between 2 feeds			
	Commercial pellet		Brine shrimp	
	Time (mins)	Entries	Time (mins)	Entries
1	7	3	7	5
2	7	4	7	4
3	7	2	7	1
4	0	0	14	2
5	6	1	7	1
6	6	3	7	2
7	5	6	9	7
8	5	6	9	5
9	6	7	8	8
10	5	5	7	7
Total	54	37	82	42

FEED FORMULATION 4

Replicate	Choice between 2 feeds			
	Bloodworm		Mosquito larvae	
	Time (mins)	Entries	Time (mins)	Entries
1	5	3	8	3
2	11	2	2	1
3	5	5	8	7
4	3	4	8	7
5	7	8	8	7
6	7	8	8	7
7	10	9	4	7
8	7	5	7	9
9	6	9	8	11
10	6	9	8	11
Total	67	62	69	70

FEED FORMULATION 5

Replicate	Choice between 2 feeds			
	Bloodworm		Brine shrimp	
	Time (mins)	Entries	Time (mins)	Entries
1	10	12	4	8
2	8	13	6	9
3	9	10	6	11
4	7	11	8	10
5	8	11	5	8
6	6	7	7	9
7	8	9	7	9
8	8	8	7	13
9	12	8	3	7
10	12	8	3	4
Total	88	97	56	88

FEED FORMULATION 6

Replicate	Choice between 2 feeds			
	Mosquito larvae		Brine shrimp	
	Time (mins)	Entries	Time (mins)	Entries
1	6	5	9	10
2	7	3	8	7
3	8	5	5	4
4	7	4	5	4
5	9	6	6	6
6	12	6	3	3
7	2	2	10	3
8	2	4	13	3
9	6	5	4	5
10	6	3	5	5
Total	65	43	68	50