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A Low Cost Amphipod-Based Feed for Rearing of Ornamental Aquarium Fish, *Poecilia reticulata* (Peters).

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Abstract

Protein is an important but expensive dietary requirement for the growth of aquarium fish. *Platorchestia platensis* (Crustacea, Amphipoda), is easily available on the shores of Mauritius, was used as a protein source in feed preparation for rearing the guppy (*Poecilia reticulata*). The effect of the amphipod based feed (AF)(containing 25% amphipod, 20% milk protein, 30% carbohydrates, 22.5% lipids, 0.5% vitamin and 2 % minerals), and that of a commercially available feed (CF) on growth was investigated. AF is 10 times less expensive than the commercial feed. From the time of birth, 100 mg of the test feed was given twice daily for a period of 10 weeks to 32 Guppies. Total length was measured at the end of each week. Feed attractiveness of the amphipod-based feed to adult Guppies was also investigated. Thirty trials were conducted in pet houses each containing five fish and behavioural responses were recorded. Mean length of the fish at 10 weeks was 30.7 ± 3.0 mm for amphipod-based feed, and 31.0 ± 4.2 mm for commercial feed. No significant difference was noted in growth while using the two feeds ($F_{(1,47)} = 0.06$, $p = 0.8$) indicating that the AF is as good as the CF. However, during the first feeding week better growth was observed using AF ($F_{(1, 61)} = 4.99$, $p = 0.03$). Behavioural experiments with adults showed a preference for powdered form of AF compared to flakes and pellets. Higher percentage ingestion was observed when using AF compared to CF.

Keywords: Guppies, Amphipod based feed, commercial feed, growth, feed attractiveness, behaviour

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1. INTRODUCTION

Fish, like all other heterotrophs, require some essential dietary constituents such as proteins, lipids, carbohydrates, vitamins and minerals for proper growth, bodily functions and reproduction. Proteins are readily usable sources of energy for fishes and they have a high degree of absorption and digestion (Philips, 1972). Proteins are mainly used in anabolic processes such as tissues production and growth and in the absence of protein, growth retardation occurs (Ashley, 1972). Although fish are able to synthesise certain amino acids readily (Lagler *et al*, 1977), 10 of the essential amino acids cannot be synthesised and have to be obtained from the diet (Craig & Helfrich, 2002). Protein is the most expensive part of fish feed (Craig & Helfrich, 2002) and with the ever-increasing price of fish protein; the aquaculture industry is looking for alternative low-cost sources of high quality proteins.
Amphipods constitute a major part of the diet of fishes (Zuckerman, 2000; Brode, 2001; Moosoohur, 2005), marine mammals (Brown and Mac Lachlan, 1994) and the great whales (Wikipedia, 2006). Due to their high abundance, both in the oceans and sandy beaches (Brown & McLachlan, 1994; Myers, 1997), the amphipods are gaining more and more attention as potential protein sources in the aquaculture industries. Amphipods are nutritious, rich in essential amino acids and contain high levels of Methionine, Astaxanthin and Omega-3 type fatty acids (Pouliot, 2004). Fish reared on a mixture of amphipods, copepods and krill show growth rates that are at least as good as when the fish are reared on first-class fish based feeds (Suontama, 2004). The addition of these crustaceans to the diet also increases appetite.
The guppy is an omnivorous fish that subsists on a diet composed of both vegetable and animal food (Skelton, 1993**)**. The foraging and feeding responses of the Guppy depend on group size (Schmidt, 2002) and presence of members of the opposite sex (Griffiths, 1996). Chemical stimuli from the feed, physical texture and particle size are the most important cues that affect feeding (Noakes & Baylis, 1990). The gape size of the fish also has an impact on the food that it will consume.

The aim of the present study was to determine the effect of a low-cost amphipod-based feed (AF) on the growth of Guppies. AF was tested against 'Fancy Guppy' (CF), a leading brand of feed for freshwater ornamental fish. The attractiveness of different textures of AF namely pellets, flakes and powder was then investigated. The same texture of AF and CF were subsequently used to determine which feed was more attractive to Guppies.

2. MATERIALS AND METHODS

2.1 Feed Preparation

The sand hopper *Platorchestia platensis* was used to make the AF. *Platorchestia platensis* is easily available on most beaches where it occurs in algal debris, dead decaying seaweed and associated sand. The amphipods were collected from two different sites namely from Pointe-Aux-Sables on the west coast of Mauritius and Anse-La-Raie in the northeast of the island. The samples of sand and algal debris were frozen at -4°C for 24 hours in order to kill the amphipods. The amphipods were removed from the substrates using forceps and were frozen at - 4°C to preserve them. When sufficient amounts were collected, they were oven-dried at 60°C and crushed to fine powder to make the feeds. The protocol for the preparation of the

amphipod feed was based on Moosoohur (2005), Bureau (2004) and Pillay (1995). The micronutrients for the study were provided free of charge in the form of vitamin and mineral premixes by a private company (Livestock Feeds Limited). However, multivitamin and mineral pills such as Centrum (Bureau, 2004) can also be used to supply the micronutrients.

Table 1: The composition of 100 g of the AF. The source and the price of the different ingredients are indicated. 100 grams of the AF costs Rs 29.76

Ingredient	**Amount (g)**	**Source of ingredient**	**Cost**
Amphipods	25 g	Collected from the sand	30 mins to collect samples and 5 hrs to separate amphipods
Milk protein	20 g	From grocery store	Rs. 2.00
Wheat flour	30 g	From grocery store	Rs. 0.21
Sunflower oil	22.5 g	From grocery store	Rs. 11.25
Vitamin premix	0.5 g	Livestock feed limited/drug store	Rs 16.30 for 2 Centrum Pills
Mineral premix	2.0 g	Livestock feed limited/drug store	

The above ingredients were mixed with water to make a dough. Pellets were made by extruding the dough through a vegetable grater. The dough was spread into thin sheets using a rolling pin to make flakes. These were oven-dried overnight at 60°C (Pillay, 1995). Powdered feed was made by crumbling the cooked dough and sieving it to obtain fine particles (Bureau, 2004.).

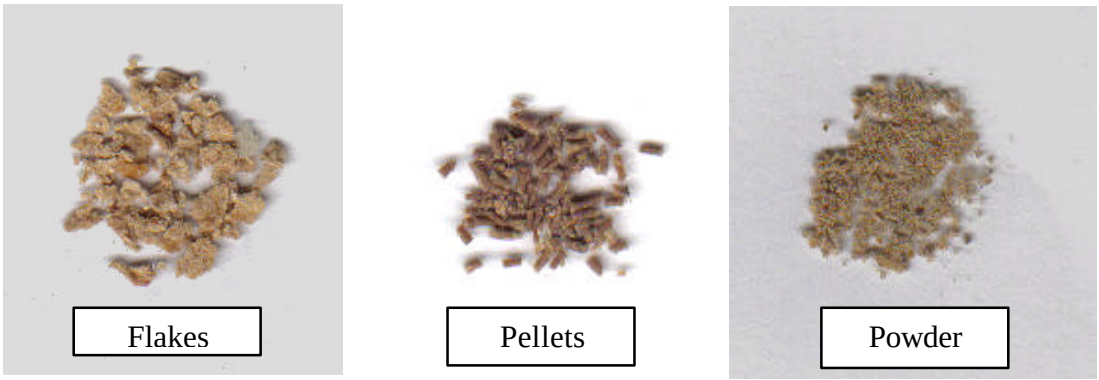

Figure 1: The different textures of Amphipod-based feeds prepared during the study. From left to right flakes, pellets and powdered forms of AF.

All the feeds prepared were placed in airtight, labelled containers and stored in a cool, dry place out of direct sunlight to maintain the quality of the feed and prevent the development of moulds (Stickney & Kohler, 1990).
The commercial feed used was Hikari Tropical 'Fancy Guppy' (Fig. 2). It was purchased from a pet shop.

Figure 2: The commercial feed 'Fancy Guppy'. It is supplied in a resealable, plastic-coated aluminium foil packet. It is in the form of micro pellets.

2.2 Experimental aquarium setup

The growth experiment was carried out in replicates running simultaneously. Four aquaria 90 x 41 x 35 cm were filled up to ¾ with tap water and labelled AF1, AF2, CF1 and CF2. Air was supplied via an aeration tube to which was attached an air stone (Landau, 1992). The aquaria were set up prior to the start of the experiment to allow the water to cycle and remove chlorine from the water (Stickney & Kohler, 1990). Every week, 50% of the bottom water was siphoned out (Blakely & Hrusa, 1989) and replaced with water that had been standing for a week.

All the fry (a total of 64) were obtained at the same time from the parental stock of fish maintained in the laboratory. Shortly after birth, 16 fry were measured and transferred to each of the aquaria and they were given the test feed from first feeding. Each day, the fish were given 200 mg of feed which corresponds to 4 % of their body weight (Stickney & Kohler, 1990). This mass was divided into two portions of 100 mg each and given at the same time each day (Busacker *et al.* 1990). Feed was usually given at 0930 hrs and 1530 hrs. The fry in AF were given the Amphipod-based feed and the fry in CF were fed the commercial feed.

2.3 Growth parameters

During this experiment, increase in total length was used as a measure of growth. The length of each fish was measured to the nearest millimetre using Vernier Callipers (Strauss & Bond, 1990) after every 7 days for the next 10 weeks. The initial length corresponds to the length of the fry just after birth. The data were used to calculate growth indices. The length measurements were tabulated and used to calculate the mean total length, the standard deviation of the mean and the relative growth rate. Relative growth rate was calculated using the following formula (Busacker *et al,* 1990)

$$\text{Relative growth rate} = \frac{Y_2 - Y_1}{Y_1 (t_2 - t_1)} \times 100$$

Where Y_1 is the initial length in mm of the fish

Y_2 is the final length in mm of the fish after 7 days

t_1 is the initial time in days

t_2 is the final time in days.

2.4 Feed texture preference experiments

The feed texture preference experiments were carried out in small 12 cm × 22 cm ×13 cm aquaria referred to as pet houses. These were half filled with standing, aerated water and five randomly selected adult fish were transferred to the pet house a day prior to the experiment. The same number of males and females was used. The length of males was 40 mm ± 6 mm while the females measured 51 mm ± 10 mm. The experiment was carried out at 1000 hrs and the fish had been fed on the eve at 1530 hrs.

At each trial, 10 mg of feed was released in the pet house and the number of fish showing any given behavioural response was recorded for the next three minutes. Five such releases were conducted on any given batch of fish, alternating between pellets, flakes, and powdered form of the AF and the CF. A total of 30 trials were conducted for each feed type (n=30). After the trials, the fish were returned to the stock aquarium and the apparatus was set up using 5 different fishes.

The feeding response of the fish was assessed according to a behavioural method developed by Stradmeyer (1989) to test the feeding response of fish. Five categories of feeding responses were recognised: Orientation: the fish shows eye and body movements in response to the presence of the feed, Approach: the fish swims towards the food source, Capture: the fish takes the feed in its mouth, Rejection: the fish spits out the feed that it had taken in and Ingestion: the fish ingests the feed.

2.5 Statistical analysis

The mean values for total length were calculated at specific weekly intervals. Data for replicates were pooled after testing for differences. Analysis Of Variance (ANOVA) Test was carried out to determine if there were significant difference between the mean growth rates as observed under the AF and the CF feed types. Before using ANOVA, care was taken to verify the assumptions of normality. A one-way ANOVA was carried out since only one variable (length) is under consideration. The test was carried out using MINITAB. The level of significance was set at 5% and a p-value of less than 0.05 would indicate significant difference between the means of the two treatments. The data obtained for the feed preference experiment was analysed qualitatively to determine if there were differences in the feeding responses of the guppy to the different feed types and textures.

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3. RESULTS

3.1 Growth experiment

A gradual increase in length of the fish was observed in both batches. However, there were differences between the length of the fish grown on the AF compared to those grown on the CF. An ANOVA test was performed to determine if these differences are statistically significant at the 5% level.

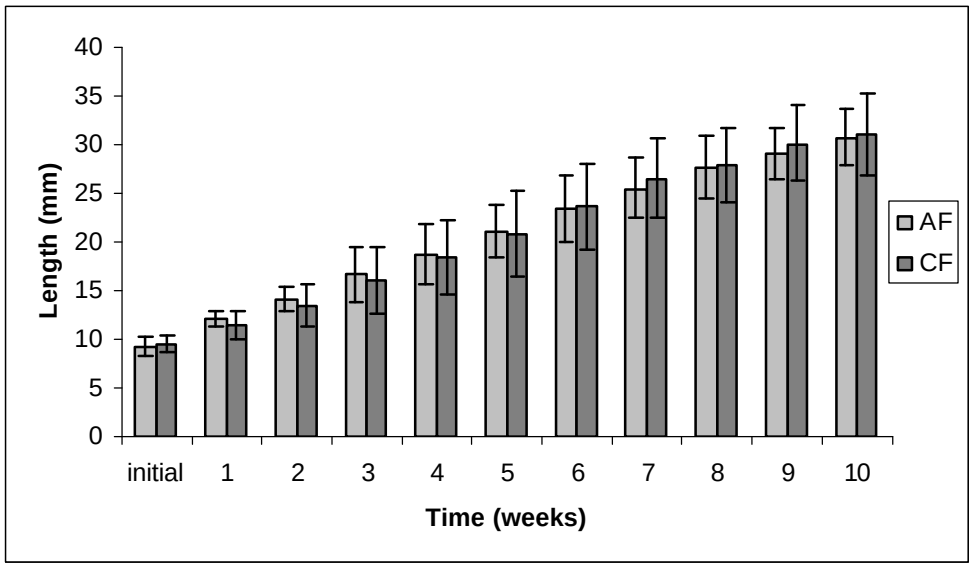

Figure 3: The mean total length of the fish (mm) in relation to time (weeks). The first value on the time axis refers to the length of the fish when they were placed in their respective aquaria.

Table 2: The mean length and the standard deviation for the Amphipod-based feed and the commercial feed. All numerical values are given to 3 s.f. The results of the ANOVA test and the remarks are included.

	length			**Test**		
Time	**AF**	**CF**	**n**	**p**	**f**	**remarks**
Initial	9.25 ± 0.986	9.45 ± 0.789	(1,61)	0.397	0.727	no significant difference
week 1	12.03 ± 0.676	11.4 ± 2.24	(1,61)	0.029	4.99	significant difference
week 2	14.1± 1.92	13.4 ± 4.76	(1,57)	0.107	2.69	no significant difference
week 3	16.6 ± 7.56	16.0 ± 11.1	(1,51)	0.469	0.532	no significant difference
week 4	18.7 ± 9.61	18.4 ± 13.9	(1,50)	0.75	0.102	no significant difference
week 5	21.1 ± 7.72	20.8 ± 19.8	(1,50)	0.771	0.086	no significant difference
week 6	23.4 ± 11.8	23.6 ± 19.0	(1,49)	0.839	0.042	no significant difference
week 7	25.5 ± 9.76	26.5 ± 17.3	(1,49)	0.34	0.93	no significant difference
week 8	27.6 ± 9.66	27.8 ± 14.8	(1,48)	0.839	0.042	no significant difference
week 9	29.0 ± 7.00	30.2 ± 15.4	(1,48)	0.224	1.52	no significant difference
week 10	30.7 ± 8.89	31 ± 17.8	(1,47)	0.802	0.063	no significant difference

The results (Table 2) show that there were no significant differences between the mean lengths of the two batches of fish at the end of the ten weeks that the experiment lasted $F_{(1,47)} = 0.06$, $p = 0.8$) showing that the low-cost amphipod-based feed used in the experiment is as good as the commercial feed. In fact, during the first week of growth, the fish reared on the AF grew better than the fish reared on the CF ($F_{(1, 61)} = 4.99$, $p = 0.03$).

The relative growth rate of the fish in both AF and CF aquaria was high (Fig 4)at the start of the experiment and decreased subsequently.

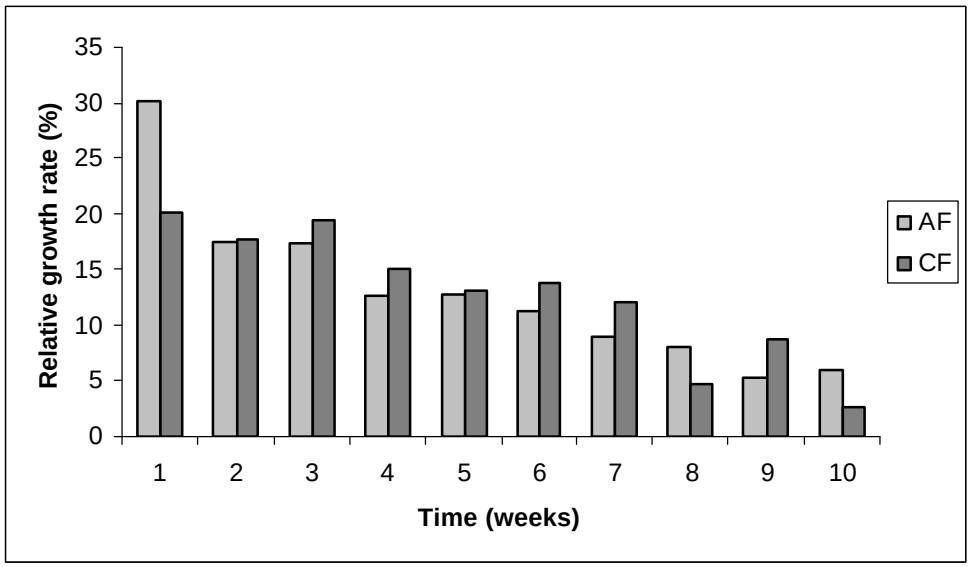

Figure 4: The relative growth rate of the fish over the ten week period. Values on the Y axis express the relative growth rate as percentages while the X axis shows time in weeks.

Guppies in both groups reached sexual maturity and produced normal offspring during the 10 week experiment.

3.2 Feed attractiveness experiment

The behavioural response experiment was carried out using the AF and the CF to determine if the fish preferred the amphipod-based feed or the commercial feed when both are presented in the same texture. The results were expressed as percentages and used to plot a flow chart. The percentage ingestion for the AF was higher than the percentage ingestion for the CF. Since both the AF and CF were presented in powdered form, the fish preferred the AF to the CF.

The feeding response of the Guppy to three different textures of feed AF was investigated. Higher percentage ingestion was observed for the powdered form of the AF. Although the fish ingested the pellets and flake form of AF, the food was often rejected.

Both feeds were presented in the same form but the proportion of Capture-ingestion was higher for the AF indicating that the fish prefer the AF more than the CF.

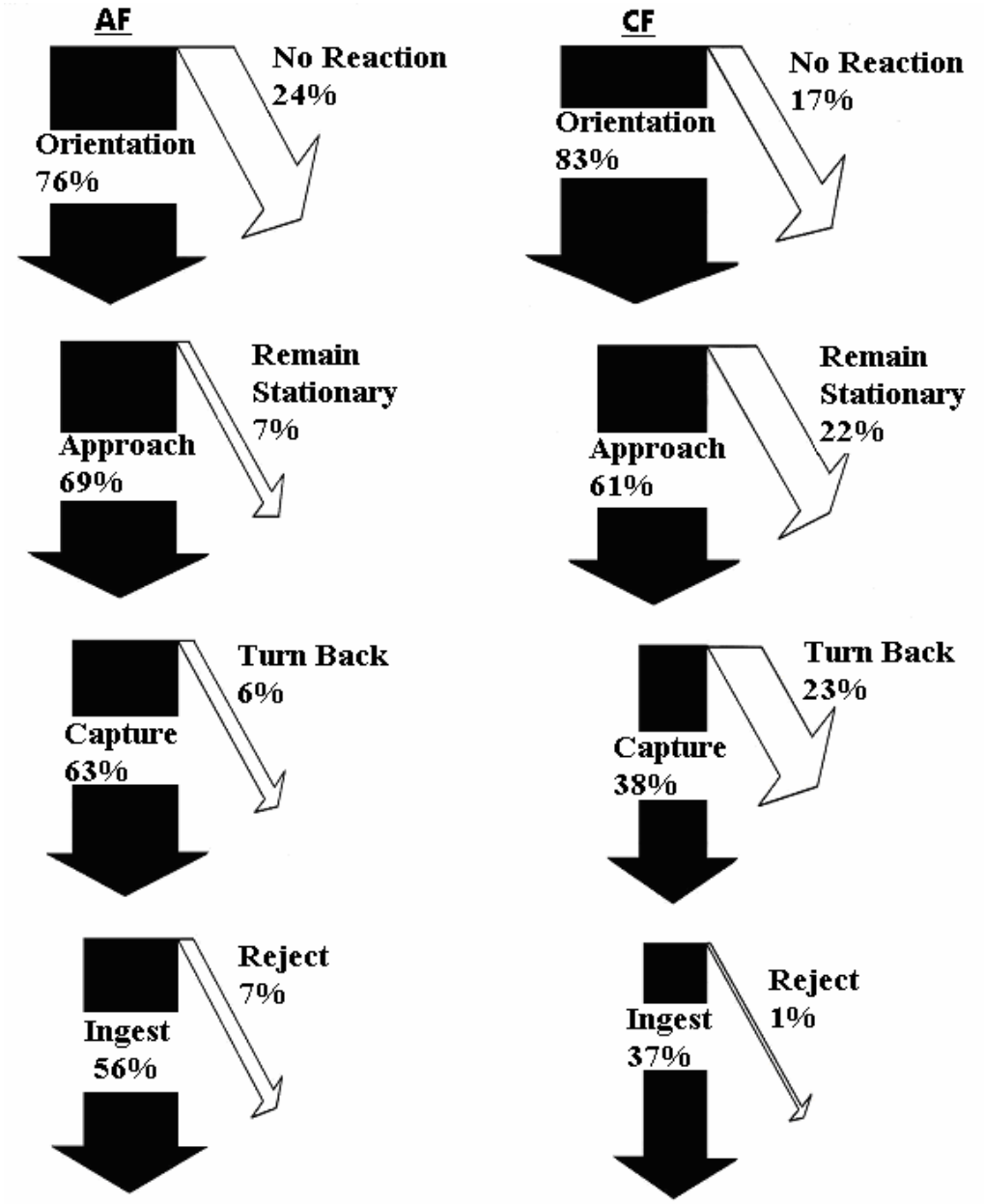

Figure 6: Comparison of the feeding responses of the Guppy to the different feeds presented. The flow chart on the left corresponds to the response elicited by the Amphipod-based feed (number of trials=30) while the chart on the right shows the responses to 'Fancy Guppy' (number of trials=30). The thickness of the arrows is proportional to the percentage of fish that show a given response.

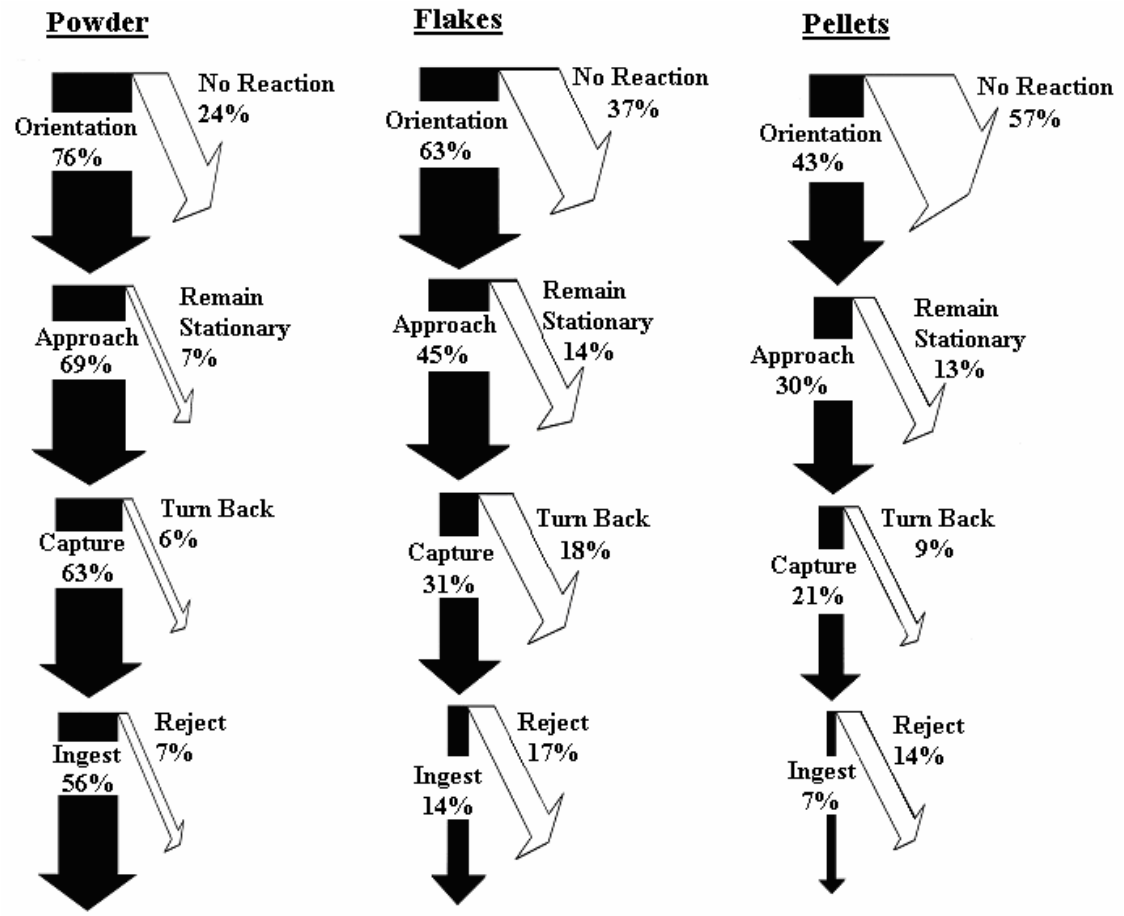

Figure 7: The behavioural response to the presence of three different textures of feed AF. The flow diagrams represent the percentages of any given response. For each texture of the AF number of trials=30.

3.3 Feed characteristic and price comparison

The cost of the AF was compared with that of different feeds for guppies. The data (Table 3) collected show that the AF is a less expensive feed for guppies. It is approximately 10 times less expensive than 'Fancy Guppy'. Comparison between the CF and the AF (Table 4) shows that the AF is similar to CF in almost all respects to 'Fancy Guppy' with the exception of a preparation time for AF.

Table 3: The different feeds for guppies available on the market. The data were collected from different pet shops. Fancy Guppy is the most costly feed while the AF is least costly.

Feed Name	Feed Description	Price for 100g
Amphipod Feed	Powder	Rs 29.76
Aquafin	Flakes	Rs 75
Aqaudene	Micro pellets	Rs 100
Yumizi	Micro pellets	Rs 100
Fancy Guppy	Micro pellets	Rs 300

Table 4: Characteristics of the commercial feed and amphipod feed

Characteristic	**Commercial feed**	**Amphipod feed**
Cost	Rs 300.00 for 100 g of feed	Rs 29.76 for 100g of feed
Texture	Powder (micro pellets)	powder
Storage conditions	Cool dry place, away from direct sunlight	Cool dry place, away from direct sunlight
Acceptability of feed	Readily fed upon by the guppy	Readily fed upon by the guppy
Preparation time	Bought from pet shop	Preparation time: 2 days

4. DISCUSSION

4.1 Growth experiment

Amphipods are available in huge quantities and are good sources of fats and proteins (Suontama, 2004; Pouliot, 2004). Amphipods are therefore gaining interest as an alternative, less expensive source of protein in formulated feeds. Fish feeds prepared with plant (soybean meal) protein, though less expensive than those prepared with high quality fish protein, are typically low in methionine; therefore, extra methionine must be added to soybean-meal based diets in order to promote optimal growth and health (Craig & Helfrich, 2002). Furthermore, carnivorous fish species are not well adapted to plant based feeds (Suontama, 2004). An amphipod-based feed does not present these problems as amphipods are rich in methionine and are more easily accepted by carnivorous fish species.

The growth rate of the fish in both treatments fluctuated, with troughs and peaks corresponding to growth spurts. According to Shaddock (2003), the growth spurts of the guppy can be attributed to the rising and falling levels of the growth hormones. The growth rate also decreased with time and this is consistent with the knowledge that the growth rate of animals decreases as they become older. This decrease in growth is controlled by the growth hormone produced by the fish and the environmental conditions (Swift, 1993).

The fish reared with amphipod feed grew as much as those reared with the commercial feed implying that AF is as good as the commercial feed. Moreover, the fish in both treatments reached sexual maturity and produced normal offspring. This indicates that AF contains all the nutrients required for proper growth in the guppy as nutritional deficiencies result in decreased growth, development and reproduction (Ashley, 1972; Halver, 1992).

The AF has the added advantage of being approximately 10 times less expensive than the CF. The ingredients used to make AF can be easily obtained from the grocery store and the amphipods collected from beach wrack (Tett, 1987; Brown & McLachlan, 1994; Myers 1997). The AF also provides Astaxanthin (Pouliot, 2004) which enhances coloration in ornamental fish. The AF is also easy to store. If it is kept in a cool dry place away from direct sunlight, its quality is maintained and molds do not develop on it (Stickney & Kohler, 1990).

4.2 Feed preference experiment

In this study, higher percentage ingestion was observed for the amphipod feed compared to the commercial feed. Other studies have shown that amphipods contain compounds such as betaine that attract fish (Regenstein & Regenstein, 1991).

The AF was more readily accepted in the powdered form compared to the pellet and flake form. Noakes and Baylis (1990) have observed that most important cues that affect feeding in fish are physical texture, particle size and gape size of fish. Guppies are small fishes that have small gape sizes and prefer small feed particles. The fish also prefer the powdered feed, because fish actively choose prey of certain sizes such that food processing is energetically most efficient (Mittelbach, 1983 in Busacker *et al*, 1990. Studies have shown that Guppy fry reared on a powdered feed show enhanced growth compared to fry reared on diets in the form of flakes (Harpaz *et al*¸ 2005). Feeding on powder instead of flakes or pellets may be energetically more efficient. There was no difference between the feeding response of female and male guppies as both male and female guppies share the same taste preferences (Nikolaeva & Kassumyan, 2000 in Magurran, 2005)

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