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A Comparative study on the antibacterial activity of Plant material incorporated feed in *Poecilia latipinna* and *Xiphophorus helleri*

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ABSTRACT

A comparative feeding experiment carried out in two ornamental fishes *P. latipinna* and *X. helleri* (*Poeciliidae* family). They were supplemented with six types of feed designated as CF (commercial feed), TD1 (Control feed), TD2 (*Allium sativum* feed), TD3 (*Bacopa monnieri* feed), TD4 (*Myristica fragrance* feed) & TD5 (Combined feed) for 60 days. After the stipulated time the survived fishes were sacrificed to isolate their tissues like gut, gill along with water samples to evaluate the bacterial load. Analysis of variance (ANOVA) test of microbial load in Aquaria revealed highest in TD1 (1475000 CFU) and lowest in TD4 (165000 CFU). The ANOVA Test showed that there is significant difference in the microbial load among different feeds. In *X. helleri* ANOVA test showed that there is significant difference in the gut mean microbial load with highest in TD1 (590000 CFU) and lowest in TD5 (98500 CFU) and its mean microbial load in gill was highest in TD1 (640000 CFU) and lowest in TD5 (15000 CFU) among different feeds at 1% level of significance (p-value < 0.01). In *P. latipinna* the mean microbial load in gut expressed higher in TD1 (490500 CFU) and lowest in TD3 (15500 CFU) and the ANOVA test showed no significant difference among feeds while the mean microbial load in the gill was highest in TD1 (640000 CFU) and lowest in TD5 (15000 CFU) with significant difference among different feeds at 1% level of significance.

Key words: *P. latipinna, X. helleri, Allium sativum, Bacopa monnieri, Myristica fragrance*

Introduction

In ornamental fish sector diet imparts a crucial role in determining their physiological and morphological aspects. It is mandatory to meet the basic requirements like stability palatability along with water quality maintenance when dry pelleted feed are offered to ornamental fishes as they are in captivity. Application of dry pelleted feed excessively distorts the hydrological parameters. A direct relationship exists between the water quality and disease outbreak which in turn ends with antibiotics. Behavioural changes, lack of feeding, white spots, haemorrhages, presence of fungi can be accounted as early signs of poor water quality. The clinical signs include poor movement, white film on the eye, rapid movement, gasping at surface, pop eye, failure in egg development as mentioned in the book entitled "Water quality in the ornamental aquatic industry, (2006)".

The deliberate administration of antibiotics harms the beneficial microbes, results in fish fries death, dwindle the lifespan of fishes and arousal of antibiotic resistant microbes. As a remedial measure we should initiate the breeders to supplement plant material incorporated feed as a biosafety measure stated in Water quality in the ornamental aquatic industry (2006). The Living Jewels (2008), a handbook on fresh water ornamental fish explained that bacterial infections arises via poor water quality,

improper nutrition, excessive parasitism either external parasites(*Dactylogyrus, Gyrodactylus, Chilodonella, Ichthyophthirius, Tetrahymena, Piscinoodinium, Trichodina, Ichthyobodo, Uronema, Lernaea* and *Argulus*) or internal parasites (Myxosporidians, Microsporidians, Nematode, Caprillaria).

It is a universal fact that entire or sections of plant impart medicinal property as they own secondary metabolites in its cells (Divyaand Sreeja, 2017). This awareness prompted me to formulate five entirely different feeds namely TD1 (Devoid of plant material), TD2 (*A. sativum* feed), TD3(*B. monnieri* feed), TD4 (*M. fragrance* feed) and TD5(Mixed plant feed). Further a comparative study carried out with reference to the commercial feed designated as CF. Microbial load especially bacterial load was evaluated as some microbes act as opportunistic pathogens while others as obligate pathogens (Durborow and Ruth Francis-Floyd 1996).

Materials and Methods

The experimental fishes like *Poecilia latipinna* and *Xiphophorus helleri* were acclimatised to laboratory conditions for 15 days were subjected to feeding experiments for 60 days by providing with six types of feed designated as CF (Commercial feed), TD1 (Control feed devoid of plant materials), TD2 (*A. sativum* feed), TD3 (*B. monnieri*), TD4 (*M. fragrance*) and TD5 (Combined plant material incorporated feed). 1gm of dried plant material was added in the respective feed to evaluate the mean microbial load among the selected fishes.

In vivo antibacterial efficacy of feed estimated using APHA, (2005)

The fishes after rearing for a period of 60 days were brought back to the laboratory. The water from the respective tanks was also collected and stored at 2^0C temperature for bacteriological analysis by isolating gut, gill and water samples. The reared fishes in the five treated groups CF, TD_1, TD_2, TD_3, TD_4 and TD_5 after commencing for an experimental period of 60 days were starved for 24 hours, paralyzed by putting in ice cold water for 15 minutes followed by surface sterilization (70% alcohol). The tissues like gut, gill from treated fishes later on isolated removed and placed in a mortar and squashed well using 1 ml of phosphate buffer. One ml of the tissue homogenate was made up with 10 ml of buffer solution to get 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} dilution. Similarly the water samples were also sorted to detect the microbial load.

Preparation of culture media

Nutrient agar medium (Agar agar: 15 g/l, Beef extract: 3 g/l, NaCl: 5g/l, Peptone 5 g/l) was used for obtaining the bacterial culture. After autoclaving the medium, allowed it to cool between 45 ^{0}C and 50 ^{0}C. A 0.3 cm thick agar plate was obtained by pouring 15 to 20 ml of media per 100× 15mm plate. A volume of 100 µl bacterial suspension from each dilution was distributed evenly over the surface of the agar plate using a smooth sterilized spreader. All the plates were incubated at 32 ^{0}C for 24 to 48 hours and evaluated the number of colonies by colony counter

Table 1. ANOVA of Mean Bacterial load in gut and gill of *X.helleri*

		N	Mean	SD	F-value(p-value)
GUT	CF	2	540000.00	14142.1	55.16(<.01)
	TD1	2	590000.00	14142.1	
	TD2	2	75000.00	21213.2	
	TD3	2	205000.00	21213.2	
	TD4	2	120000.00	14142.1	
	TD5	2	98500.00	101116.3	
	Total	12	271416.67	223733.8	
GILL	CF	2	327000.00	386080.3	5.95(<.05)
	TD1	2	715000.00	35355.3	
	TD2	2	30000.00	28284.3	
	TD3	2	120000.00	28284.3	
	TD4	2	30000.00	14142.1	
	TD5	2	20000.00	14142.1	
	Total	12	207000.00	287248.4	

Results and Discussion

The data were subjected to One way analysis of variance (ANOVA) statistically using SPSS version 16.0. The data revealed that mean bacterial load in gut was highest in TD1 (590000CFU) and lowest in TD5 (98500CFU). The ANOVA test showed significant difference in microbial load among different feeds at 1% level of significance (p-value<.01). The ANOVA test showed significant difference in microbial load among different feeds at (p-value<.05) (Table 1).

Mean bacterial load in Gut and gill of *P. latipinna*.

The mean microbial load in the gut was highest in TD1 (490500CFU) and lowest in TD3 (15500CFU). The ANOVA test (Table 2) showed no significant difference in microbial load among different feeds (p-value>.01). The mean microbial load in gill was highest in TD1 (640000CFU) and lowest in TD5 (15000CFU). The ANOVA test showed significant difference in microbial load among different feeds at 1% level of significance p-value<.01).

The mean microbial load in aquaria was highest in TD1 (1475000CFU) and lowest in TD4 (165000CFU). For TD2 (210000 CFU), TD3 (205000 CFU), TD5 (180000 CFU) and CF (1175000 CFU) the microbial load obtained were The ANOVA testhence showed that there is significant difference in microbial load among different feeds at 1% level of significance (p-value<.01).

Discussion

Through my work I aimed to emphasize the need to implement natural plant ingredients as an alternative to synthetic chemicals for sustainable development in the ornamental fisheries sector. Abdelwahab and El- Bahr (2012), Poongodi (2012), Bhavan *et al.* (2014) studied the application of various plants like *Zingiber officinalis, Curcuma longa*, *Ricinus communis*. Abdelwahaband El- Bahr (2012) detailed about the Serum biochemistry of *Lates calcarifer* increased by the application of *Nigella sativa* and Turmeric mixture. *Trigonella foenum graecum*, Fenugreek sprouts Med, fresh leaves of Eucalyptus, hot pepper meal, thyme seed meal (*Thymas vulgaris), Matricaria rucetita* augmented growth performance in Nile Tilapia accounted by Hayam *et al.* (2011). The incorporated diets with *Allium sativum* (Garlic), *Bacopa monnieri* (brahmi), *Myristica fragrans* (nutmeg) and its combination accelerated survival with better feeding rate. The weight gain in test diets increased compared to CF and TD1 like feeding with *Trigonellafoenum-graecum* (Fenugreek) in *Macrobrechium rosenbergii* by Poongodi (2012).

The medicinal curative property of *A. sativum, B. monnieri* and *M. fragrans* rescued *X.heleri* and *P. latipinna* in agreement with Lovatelli and Chen (2009). It was clear that the assessed values of microbial content remained excellent in TD2, TD3, TD4 and TD5 than CF and TD1 noticed throughout our experiment with respective fishes. He also studied effect of garlic (*Allium sativum)* feed in sword tail (*Xiphophorus helleri*) resulted improved growth performance, survival rate, and bacteriological characteristics also goes in conformity Jegede Temitope, (2012). The in vivo antimicrobial efficacy of *C. asiatics* supplement in *M. rosenbergii* showed a sig-

Table 2. Mean Bacterial load in gut and gill of *P.latipinna*

		N	Mean	SD	F-value(p-value)
GUT	CF	2	70000.00	1414.21	1.26(.388)
	TD1	2	490500.00	564978.32	
	TD2	2	125000.00	7071.07	
	TD3	2	15500.00	707.11	
	TD4	2	16000.00	1414.21	
	TD5	2	40000.00	14142.14	
	Total	12	126166.67	243993.60	
GILL	CF	2	580000.00	14142.14	315.5(<.01)
	TD1	2	640000.00	56568.54	
	TD2	2	30500.00	2121.32	
	TD3	2	21500.00	2121.32	
	TD4	2	25000.00	7071.07	
	TD5	2	15000.00	7071.07	

Mean bacterial load in the aquaria water samples

nificant reduction in the pathogenic bacteria loads approved the application of selected plant materials (Salini *et al.,* 2013).

It was the phytochemicals in *A.sativum*, *B.monnieri* and *M.fragrans* that amplified growth and survival in *X.heleri* and *P.latipinna* like the application of *Centella asiatica* by reducing the microbial content.The antimicrobial efficacy of the feed hence evaluated strengthened the antibacterial nature of the feeds justified by Poongodi (2012). Our findings thereby agreed the findings of Salini *et al*. (2014) who assessed the in vivo antibacterial efficacy in *M.rosenbergii*. Secondary metabolites improved survival in experimental fishes due its higher PER. Herbal substituents offered protection against diseases than the commercial fed groups was also suggested by Hashim, (2011). On conclusion arrived at the fact that secondary metabolites in these experimental plants own the ability to reduce the harmful microbial load in tissues and aquaria compared to Control feed and Commercial feed.

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