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Application of *Myristica fragrans* feed in *Poecilia latipinna* as an effective antibacterial agent and colour enhancer

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Abstract: *Myristica fragrans* seed (Nutmeg) famed as the prominent fatty seed of medicinal and therapeutic quality came from the Spice island of Indonesia. It is endowed with phytochemicals, minerals and vitamins impart medicinal values. In captive conditions the ornamental fishes with deprived nutritional feed subjected to diseases and faded colour produces economic loss in their trade operations can be tackled by effective administration of plant products. In this perspective, a comparative study made in *Poecilia latipinna (*yellow molly*)*by providing (commercial feed) CF and TD_1 (*Myristica fragrans)* for a duration of 60 days. The in vivo antibacterial efficacy of feed in tissues(gut & gill) of the specimen along with water samples from aquaria revealed positive result for TD_1.The colour enhancing ability in yellow molly yielded best result higher for TD_1 considered to CF. The total bacterial load in the water, gut as well as the gill of CF fed group was $75x10^4$ CFU/ml, $45x10^4$ CFU/ml& $23x10^4$ CFU/ml while for TD_1 it was $14x10^4$ CFU/ml, $9x10^4$ CFU/ml, $7x10^4$CFU/ml. The quantitative estimation of carotenoids estimated was 6.32µµg/g wet weight in control and 12.12µg/g wet weight for TD_1.From the present investigation arrived at the conclusion that TD_1 provided better anti-bacterial activity than CF (commercial feed) not only prevent disease outbreak but also produce vibrant skin colour.

Keywords: *Myristica fragrans*, *Poecilia latipinna*, nutritional feed, in vivo antibacterial efficacy, carotenoids, bacterial load.

INTRODUCTION

Ornamental fishes with pleasing movement alleviate stress and pressure encountered by modern people irrespective of their residence in rural or urban areas.

The increasing awareness among people facilitated its up keeping a glorious event. Among the species currently involved in trade operations, the livebearer yellow molly (*Poecilia latipinna*)in the genera *poecilia* is a native of South eastern North America from Carolinas to the Gulf coast of Southern Mexico, dwell in fluctuating temperature and saline nature. Major attributes influencing the price value of yellow molly is healthiness with bright colour. Feeding of ornamental fishes entirely with nutrient deficient live feed in filthy nature induces diseases emphasised the supplementation of formulated feeds in the form of pellets or flakes along with living forms and pigments associated with plants, algae etc [1-3]. The commercial acceptance of fishes depends on pigmentation and disease resistance. A mutual relationship exists between the carotenoids and physiological events in fishes [4].

Breeders in an urge to utilise profit started implementing synthetic pigments and antibiotics resulted adverse environmental issues. The common agents of stress (poor hydrological parameters, rough handling, exposure to sunlight, unbalanced feeding practices etc.) promoted the arrival of diseases intend the breeders to implement medications beyond limits, resulted antibiotic resistance strains [5&6]. To wipe out such alarming situation, disease resistant brightly pigmented fishes through balanced dietary enrichment of plant materials should be generated. Thus considering the limited application of medicinal plants in ornamental fisheries sector our work aiming to explore the efficacy of *Myristica fragrans* (nutmeg seed) in preventing diseases with vibrant pigments.

MATERIALS AND METHODS

The indoor study carried out in two glass aquaria having a capacity of 30L. The yellow molly fishes (*P. latipinna)* bought from a retail shop named Achu's aquarium, Kollam district, Kerala acclimatized for 15days, fed with 3% of commercial feed twice in a day. There after fishes provided a feed weighing 3%of their body weight with experimental feeds designated as

CF and TD_1 whose proximate analysis carried out by standard methods denoted in Table-1. The fishes were well monitored with standard water quality parameters [7]. After the proclaimed experimental period (60 days), the survived fishes were collected to assess the bacterial load in the gill, gut and water samples by serial dilution method [8] with bacterial morphological characters [9].The total carotenoid content in the muscle of fishes calculated by pigment extraction method using Spectrophotometry [10].

RESULTS AND DISCUSSION

The hydrological parameters of water were well monitored during the experimental period with water temperature variance from 23^0C-26^0C. The pH fluctuated between 7.1-8.3, dissolved oxygen between 4.6 mg/l to 4.9 mg/l with a total hardness ranged from 95-114mg/l of $CaCO_3$ while the level of ammonia, nitrite and nitrate values were feeble to detect. The proximate analysis (Table 1) of feed per 100 gm of CF & TD1 revealed the composition of moisture, crude protein, crude fibre, crude lipid, ash and NFE.

Table-1: showing the proximate chemical analysis of feed in 100 g

Feed parameters	CF	TD_1(1g nutmeg)
Moisture %	12.9	11.1
Crude fibre %	2.1	2
Crude fat %	3.5	1.4
Crude protein %	26.8	30.2
Ash %	14.9	12.63
NFE %	39.8	42.67

The obtained hydrological parameters were accurate for the rearing of fishes coming under *poeciliidae* and the palatable nature of feed revealed the consumption nature of plant materials by live bearers. The retarded bacterial load in the gut, gill and water of TD_1 fed fishes clarified the anti-bacterial potency of the nutmeg incorporated feed hence agreed the presence of phytochemicals as a biosafety measure in the plant kingdom against microbes [11&12]. Thus the incidence of some common diseases like dropsy, ulcer, fin rot, tail rot, gill rot, septicaemia by *A. hydrophila* [13] can be prevented in advance revealed by using sterile disc method against it in various solvents [14 &15]. Thus it can be concluded that the presence of phytochemicals hindered the growth of bacterial species in TD_1 compared to nutmeg devoid feed CF shown in Table-2 with morphological appearance in Table-3.

Table-2: showing the microbial load (CFU/ml) in the aquaria and gut of CF & TD_1

Feed	Water(CFU/ml)	Gut(CFU/ml)	Gill(CFU/ml)
CF	$75X10^4$	$45X10^4$	$23X10^4$
TD_1	$14X10^4$	$10X10^4$	$7X10^4$

Table-3: showing the morphological features of bacterial colonies isolated from water, gill and gut of CF &TD_1 fed fishes

Bacterial species	Margin	Colony shape	Size	Opacity	Elevation	Surface	Color	Texture
W1	Undulate	Irregular	1mm	Transparent	Raised	Smooth	White	Sticky
W2	Entire	Round	2mm	Opaque	Raised	Rough	White	Not viscous
W3	Undulate	Round	3mm	Transparent	Raised	Smooth	Yellow	Sticky
W4	Entire	Round	2mm	Opaque	Raised	Smooth	Light Yellow	Viscous
W5	Undulate	Irregular	3mm	Opaque	Raised	Rough	Buff	Non sticky
W6	Entire	Round	2mm	Transparent	Flat	Smooth	Pale brown	Sticky
W7	Entire	Round	1mm	Transparent	Raised	Dull	White	Non sticky
W8	Entire	Round	3mm	Opaque	Raised	Smooth	Buff	Viscous
W9	Undulate	Irregular	1mm	Transparent	Flat	Smooth	White	Brittle
W10	Entire	Round	2mm	Opaque	Raised	Smooth	Pale yellow	Sticky
W11	Entire	Round	4mm	Transparent	Raised	Smooth	White	Brittle
W12	Entire	Round	3mm	Transparent	Flat	Smooth	Yellow	Not viscous
W13	Undulate	Irregular	3mm	Opaque	Raised	Rough	White	Brittle
W14	Entire	Round	1mm	Transparent	Raised	Smooth	Yellow	Not viscous
W15	Entire	Round	2mm	Opaque	Flat	Dull	Buff	Sticky

The extraction of carotenoids provided a better result of 12.12 µg/g wet weight in TD_1containing nutmeg than CF with 6.32 µg/g wet weight thereby approved the existing research findings. Like the application of china rose (*Hibiscus rosasinensis*) as a natural source of carotenoid in feed enhanced the pigmentation in gold fish (*Carassius auratus*)[16]. Marigold petal (*Tagetes erecta*) improved skin colour in red sword tail and tiger barb[17] while a combination of carrot (*Daucus carota*), china rose petal(*Hibiscus rosasinensis*), marigold petal (*Tagetes erecta*) and rose petal(*Rosa sinensis*) in *Amphiprion ocellaris*[18] nutmeg also with a calibre of enhancing colour. The present work also agrees the colour enhancing ability of three botanical additives coriander, Mint and Amaranth leaves on gold fish [19] Thus it can be inferred from the conducted study that the incorporation of nutmeg feed can accelerate the entire physiological functions in a vertebrate group like fishes [20].

CONCLUSION

From the study conducted it can be concluded that nutmeg supplementation in ornamental fish feed, being environment friendly, need to be promoted in future to reduce the bacterial load with vibrant pigmentation in ornamental fishes.

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