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EXPLORATION OF GROWTH AND SOME HEALTH STATUS INDICES OF Trichopodus trichopterus FED AT VARIED FEEDING REGIME

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Abstract

The health status of fish in captivity has been shown to be an important indicator to the wellbeing and life span of fishes, especially in the ornamental fish industry. Feed, feeding technique and water quality plays a pivotal role in optimizing the overall well-being of the fish. This study assessed the effect of the feeding regime on health status indicators in Trichopodus Trichopterus. Juveniles (3.6±0.2g) were stocked at 5 fish per tank in 12 aquarium tanks. They were fed to satiation at different feeding regime (twice daily) (T1), once daily (T2), once in two days (T3), or once in three days (T4) for 12 weeks. At the end of the study, body weight, survival, cortisol, glucose and antioxidant activities were measured following standard procedures. The result showed that T2 had the highest feed intake (0.78±2.18 g and 0.72±2.01g) in the second and third months, respectively. The highest final weight of 10.7±0.1g was recorded in T1 while survival was best in T2(93.3%) at 12 weeks. Glutathione activity was highest in T4 (27.12±1.18u/mg) while the least is in T2. Cortisol has the highest activity in T4 (4.7±.03) while the least was in T1 (4.02±0.12). Malondialdehyde showed a high activity level in T4 but was not statistically significant. It shows that an improper feeding regime exacts some level of stress on the fish which may lead to the loss of this precious pet over time hence pet fish keepers should put measures in place that ensures the fish feeds at least once daily.

Keywords: Fish, cortisol, glucose, antioxidants, fish health.

Introduction

Trichopodus trichopterus is one of the most repute ornamental fish among the eight native species of Indian gourami and very popular with aquarists. It is commonly known as a three spot gourami. It has high demand in the international market due to its brilliant and beautiful colour. It has also been considered as eatable fish due to its good taste but it's generally classified as a weed fish (Mandal and Barman, 2014; Sutradhar et al., 2016). Gourami is a tropical fresh water fish, found in freshwater swamps and wetlands. Gourami is attracted to areas with heavily vegetated lowlands, with temperature ranging between 24 and 30°C where they can hide (Haridas, et al, 2019). three-spot gourami has made its entry in ornamental fish markets of Nigeria. It has moderate demand among the aquarium

fish hobbyist due to its brilliant colour pattern (Paul and Chanda, 2014).

Feeding is an important facet of fish keeping, especially when fish are raised in an intensive or semi-intensive system. This has implications for the cost of fish, water quality and wellbeing of the fish. Feeding in terms of quality and quantity has implications on the overall health of fish leading to death when poorly managed. The cost of feeding depending on the purpose of the culture may be up to 60% of the total cost of production (Fagbenro et al., 2005, Aderolu et al., 2010).

Fish health is very important in every aspect of fish culture, but in ornamental fish culture fish health sometimes poses a lot of challenges both emotional and physical, because the owners of the fish develops a relationship with the fish in their tanks, over time it becomes a part of their lives, as a pet hence, when they lose their fish, it has some emotional stress on them. Often time this loss of fish is related to improper management. In some cases, the fishes are starved for days due to work schedules and engagements. Over a period, these fish develop some challenges and may end up dying. This has become a source of concern to some fish keepers, who are losing a dear fish. There are limited studies on the implications of feeding regime on health status indicators in *T. trichopterus*. Knowledge of the effects of different feeding regime on the health status can give insights on the best approach to maintaining fish well-being and development. Furthermore, it will guide fish keepers on the optimal feeding practice that will improve the quality of life and longevity of this fish species in captivity. Hence this study evaluates the implications of different feeding regime on the health status of *T. trichopterus*.

MATERIALS AND METHODS

Experimental setup

The experiment was carried out in the Department of Fisheries and Aquatic Resources Management, Michael Okpara University of Agriculture Umudike, Nigeria. The fish(3.6±0.2g) and feed (1mm) was purchased from a reputable aquarium shop. The experiment was carried out in 12 aquaria tanks(60L) with 4 treatments and 3 replicates in a completely randomized design. The aquaria tanks were stocked at 5fish per tank in triplicates. Fifty percent of the culture water was changed in every two days

Feeding regime

The fish was fed to satiation with different feeding regimes, Treatment one (T1, control) was fed twice daily. Treatment two (T2) was fed once daily, Treatment three (T3) was fed once in two days and Treatment four (T4) was fed once in three days The feeding trials was carried out for 12 weeks and water was changed weekly in all treatments. At the end of feeding trials, fish samples were taken to the laboratory for further analysis.

Test for cortisol

The fish was captured and allowed to rest in isolation for 1hour. Afterwards, the specimens was anesthetized with MS-222 (100 mg/L) and immediately after, the body part(tissue) was sampled. The sample was homogenized on ice in ethyl acetate and centrifuged for 7min at 4000rpm. The homogenate was extracted and snap frozen (Yeh et al., 2013). Samples were shared in micro titer plate with cortisol antibody coated plate and Horse Radish Peroxidase(HRP) cortisol conjugate was added. The sample was incubated and washed. The 3,31 51 51 – tetramethybenzidine(TMB) was added. The reaction was stopped using sulphuric acid. The absorbance was measured at 450nm using spectrophotometer.

$$Catalaseactivity(kU/L) = \frac{Abs(sample) - Abs(Blank1)}{Abs(Blank2) - Abs(Blank3)} \quad (1)$$

Controls: Blank 1 contained 1.0 mL substrate, 1.0 mL molybdate, and 0.2 mL serum; Blank 2 contained 1.0 mL substrate, 1.0 mL

molybdate, and 0.2 mL buffer; Blank 3 contained 1.0 mL buffer, 1.0 mL molybdate, and 0.2 mL buffer.

$$TBARS(nmol/mgprotein) = \frac{Absorbance}{\varepsilon \times L} \quad (2)$$

where $\varepsilon = 1.56 \times 10^5$ mol/L/cm is the molar extinction coefficient of MDA, and L is the path length.

Table 1: Monthly feed intake of the fish

Months	**T1**	**T2**	**T3**	**T4**
1	0.50 ± 0.07^a	0.52 ± 0.07^a	0.42 ± 0.44^a	0.28 ± 0.38^b
2	0.43 ± 0.03^b	0.78 ± 2.18^c	0.38 ± 0.39^b	0.24 ± 0.39^a
3	0.48 ± 0.02^f	0.72 ± 2.01^e	0.30 ± 0.05^{fg}	0.22 ± 0.03^g

* Means with similar superscripts across the rows are not significantly different at $p > 0.05$.

The initial weight of the fish was 3.6±0.2g. T1 recorded 6.8±0.4g, 9.8±0.02g and 10.7±0.1g in months 1, 2 and 3 respectively. T2 recorded 9.7±0.1 g and 10.4±0.05 g for second and third month respectively. T3 had 8±0.01 g and 8.5±0.01g while T4 recorded 7.1±0.2 g and 7.9±0.01 g in the first and second month respectively. T1 had the highest weight though not significantly different from T2 but was significantly different from T3 and T4. The least weight was recorded in T4 as 5.6±0.02, 7.1±0.2 and 7.9±0.1 for months 1, 2 and 3 respectively which was statistically different from other treatments. Generally, there was an increase in weight in all the treatments over the months, although month 2 showed a sharp increase in weight in all treatments.

Table 2: Monthly weight of *T. trichopterus* fed at different feeding regimes

Weight	**T1**	**T2**	**T3**	**T4**
Initial wt	3.6 ± 0.2^a	3.6 ± 0.2^a	3.6 ± 0.2^a	3.6 ± 0.3^a
Month 1	6.8 ± 0.4^a	6.7 ± 0.07^a	6.0 ± 0.1^b	5.6 ± 0.02^b
Month 2	9.8 ± 0.02^a	9.7 ± 0.1^a	8.0 ± 0.01^b	7.1 ± 0.2^b
Month 3	10.7 ± 0.1^a	10.4 ± 0.05^a	8.5 ± 0.01^c	7.9 ± 0.1^c

* Means with same superscripts are not statistically different ($P > 0.05$) across the rows.

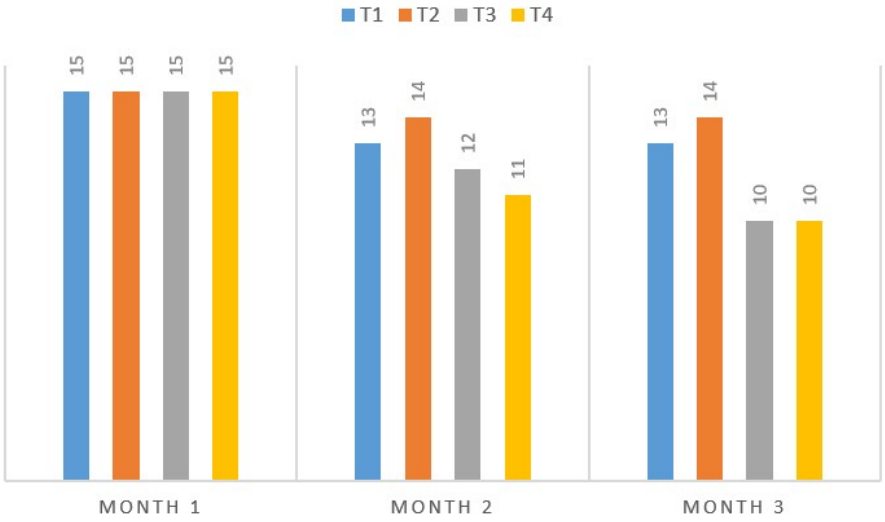

Figure 1: Survival rate of T. trichopterus fed at different regime for 3months

The result of the health indicators are shown in table 3. Glutathione had the highest activity in T4 with 27.12±1.18u/mg followed by T3 (25.36±1.20u/mg) while the least concentration was in T2 (22.46±0.85u/mg). T3 has the highest SOD concentration with 8.52±0.47u/mg but not statistically different from T4 while T2 (7.34±0.23u/mg) was the lowest activity. Glucose has its highest concentration in T1 (68.33±3.06mg/dl) while T2, T3 and T4 have, 65.33±4.73mg/dL, 66.33±3.22mg/dl and 61±2.65mg/dl respectively which was statistically difference (p0.05.) The highest level of cortisol was recorded in T4 (4.7±.03) while the least was in T1 (4.02±0.12) which was significantly different at p0.05. Generally, the cortisol activity showed a decreasing trend from T1 to T4.

* Means with the same superscript in a column are not significantly different at $p > 0.05$.

Table 3: Health indicator levels in different feeding regimes

Treatment	Glutathione (u/mg tissues)	SOD (u/mg tissues)	Catalase (u/mg tissues)	MDA (nmol/mg protein)	Glucose (mg/dl)	Cortisol
T1	25.3 ± 0.61^{ab}	7.88 ± 0.21^{c}	13.32 ± 0.64^{a}	1.27 ± 0.07^{a}	68.33 ± 3.06^{g}	4.02 ± 0.12^{a}
T2	22.46 ± 0.85^{b}	7.34 ± 0.23^{c}	12.42 ± 0.35^{a}	1.11 ± 0.08^{a}	65.33 ± 4.73^{f}	4.16 ± 0.07^{a}
T3	25.36 ± 1.20^{ab}	8.52 ± 0.47^{b}	13.89 ± 0.29^{a}	1.32 ± 0.04^{a}	66.33 ± 3.22^{f}	4.34 ± 0.14^{ab}
T4	27.12 ± 1.18^{a}	8.47 ± 0.30^{b}	14.25 ± 0.62^{a}	1.57 ± 0.09^{a}	61.00 ± 2.65^{e}	4.70 ± 0.03^{b}

* Means with the same superscript in a column are not significantly different at $p > 0.05$.

A correlation matrix was carried out between the health status indicators and feed intake as shown in table 4. The feed intake had a significant correlation with glutathione and catalase at P0.05. This implies that increase in feeding increases catalase and glutathione activity. MDA also showed a negative activity with catalase, SOD and glutathione although not statistically different. The correlation of glucose with other antioxidants showed a negative relationship which was not significantly different at p0.05. Cortisol activity showed a negative relationship with glutathione while catalase showed a significant positive relationship with glutathione.(Table 4)

Table 4: Correlations among the fish health indicators and feed intake

	Gluta	**SOD**	**Catalase**	**MDA**	**Glucose**	**Cortisol**	**Feed**
Gluta	1						
Sig. (2-tailed)							
SOD	0.760	1					
Sig. (2-tailed)	0.240						
Catalase	0.959*	0.889	1				
Sig. (2-tailed)	0.041	0.111					
MDA	-0.363	-0.463	-0.542	1			
Sig. (2-tailed)	0.637	0.537	0.458				
Glucose	-0.441	-0.126	-0.460	0.810	1		
Sig. (2-tailed)	0.559	0.874	0.540	0.190			
Cortisol	-0.093	0.523	0.081	0.122	0.667	1	
Sig. (2-tailed)	0.907	0.477	0.919	0.878	0.333		
Feed	0.988*	0.794	0.983*	0.503	0.533	0.087	1
Sig. (2-tailed)	0.012	0.206	0.017	0.497	0.467	0.913	

* Correlation is significant at $p < 0.05$ level (2-tailed).

DISCUSSION

The result of the study showed that the fish fed twice a day gave the best performance in body weight than the other fish in other treatments but not statistically different. This is in line with the work carried out by Ademola et al., (2017) where catfish was fed at different feeding regimes which showed that the control had the best mean weight. Several studies have shown that increased feeding regime has effect on the growth and water quality of the cultured fish (Ikwuemesi et al., 2018; Yang and Kim, 2019; Hamed et al., 2021). The survival rate showed that those fed to satiation twice (T1) and once (T2) a day had less mortality in the second and third month while the highest was in those feed every other day (T3) or every two days (T4). These findings are in tandem with the study by Imentai et al., (2020) which assessed the effects of feeding regime on survival of pikeperch and reported that those with increased feeding had better survival rate. In an earlier study, Deng et al., 2003 posited that overfeeding or underfeeding could result in increased fish disease and mortality, hence it is important to design a feeding regime that optimizes the fish wellbeing.

Glucose is an important source of energy in fish, especially in the brain which obtains most of its energy from carbohydrates. The normal glucose level of fish ranges between 40-90mg/dL. The result of this study reveals that the glucose level of all the treatment falls within the normal level though the control has the highest glucose level compared to other treatments. Under food deprivation, glucose requirement are satisfied by glycogen depletion to glucose or by gluconeogenesis. The balance between glucose storage and production is of utmost importance for maintaining glucose homeostasis (Polakof et al., 2012) and this explains the findings of this study. Food deprivation has been recorded to decrease plasma glucose level in fish without altering glucose uptake in the brain (Polakof et al., 2012). This agrees with the level of glucose in the food deprived treatments in this study. The fishes feed more regularly had higher antioxidants activities than those fed less frequently which follows the trend of the findings of Gao et al., (2022.) These authors in their study revealed that Takifugu rubripes fed at different feeding frequencies had no effect on the activity of SOD and glutathione. The findings of El-Araby et al., 2020 suggested an increase in the antioxidants in Nile tilapia

fed less frequently and the fish showed some degenerative histological changes in the liver. This agrees with the activity of MDA in this study although it was not statistically different from those feed more regularly.//

Cortisol is a glucocorticoid (steroid) hormone released from the adrenal gland in response to adrenocorticotropic hormones (ACTH). ACTH is a hormone released from the pituitary gland in the brain. The use of cortisol to assess the effect of stress on growth, sex, physiology and behavior has been studied (Geffroy and Bardonnet, 2016; Olivotto and Geffroy 2017; Sadoul and Geoffroy, 2019). In the study carried out by Wong et al., (2023) in dolphin, they concluded that cortisol activity as one of the stress hormone, along with other behavioral observations will aid in determining the well-being of animals. Cortisol secretion is mostly activated by various stressors which triggers the reserved energy by glycolysis (Sadoul and Geoffroy, 2019). These are in tandem with the finding of this study. In accordance with the findings of Dan et al., (2021), the effects of intelligent feeding method on the growth, immunity, and stress of juvenile micropterus salmoides revealed that basal cortisol concentration varied as a result of stress level incurred during feeding. Van der Boon et al., (1991) reported a decreased growth rate with an increase in cortisol level which agrees with the findings of this study. It has been noted that the regular negative response of cortisol on the hypothalamus-pituitary line may compromise the capacity of the fish to react to additional stress, which may lead to animal loss (Barton et al., 1987). The level of cortisol increased with increase in glucose activity which agreed with the findings of Grutter and Pankhurst (2005). They investigated capture and handling stress on Hemigymnus melapterus and discovered increased cortisol and glucose with increased stress factor and the reverse with elimination of stress factor.

CONCLUSION

Feeding regime is a very important aspect of fish keeping due to the fact that it impacts the physiological status of the fish. The study show that feeding once or twice a day showed increased growth, survival and improved well-being. Improper feeding regime such as feeding less than once a day leads to a buildup of stress hormones that may affect the health of the fish negatively, disorganise normal body functions and even lead to death over a period of time. It is recommended that T. trichopterus keepers put measures in place that will enable the fish to feed at least once daily for optimal performance and survival.

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