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EFFECT OF DIETARY L-TRYPTOPHAN SUPPLEMENTATION ON GROWTH AND SURVIVAL OF STRIPED MURREL, *CHANNA STRIATUS* (BLOCH, 1793) FRY

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ABSTRACT

Lack of quality seeds, attributed to several reasons, remains a major bottleneck in the development of wide scale farming of Striped Murrel, *Channa striatus*. A 45-days study was conducted to evaluate the effect of L-tryptophan supplemented diets on growth, survival and cannibalism of *C. striatus* fry. Twenty five numbers of fry (0.19 ±0.001 g) were stocked in aerated FRP tanks (100-L capacity) following a completely randomized design consisting of four treatments with three replicates each. L-tryptophan was supplemented in the diet of fry at 0.6% (T_1), 1.2% (T_2) and 1.8% (T_3) and a control was maintained. Fishes were fed at *ad libitum* thrice a day. The results showed that tryptophan supplemented diets had significantly higher survival rate than control group ($p<0.05$). However specific growth rate and weight gain (%) of fishes fed with tryptophan at varying levels were significantly lower compared to control group ($p<0.05$). Higher survival (84.00±2.31%) and lower cannibalism (30.67 ±4.81%) coupled with high growth (1.49±0.01g) was recorded in T_1 (0.6%) group. Based on the results of the study, it is recommended to supplement 0.6% of tryptophan in larval diet to reduce cannibalism and improve survival of striped murrel fry, although the growth was compromised.

KEYWORDS: *Channa striatus*, fry, L-tryptophan, Growth, Survival.

INTRODUCTION

In India, murrels are considered as a potential candidate species for aquaculture owing to its delicacy, unique taste, lucrative market value and their ability to withstand adverse water conditions (Kumar and Mohanty, 2018). *Channa striatus* (Bloch, 1793), popularly called as “striped murrel” in India, has good consumer preference in different parts of the country particularly in Madhya Pradesh, Bihar, Uttar Pradesh, Haryana, Andhra Pradesh, Karnataka, Tamil Nadu and all North-East States. It has gained importance in farming because of its good taste, excellent nutritional profile, air-breathing nature, rich lipo-protein content and therapeutic and market values (Kumar and Mohanty, 2016). Although the species fetches high price, the demand supply gap is very high. The major bottleneck associated with this species is the low larval survival which may attribute to cannibalism during the seed rearing as the size heterogeneity increases. In consequence, there is an imperative to solve the dilemma of low survival that may occur due to heavy cannibalism, inadequate food, improper feeding regime, stocking density and rearing conditions.

In case of predatory fish seed rearing, intra-cohort aggression and cannibalism has a major effect on survival (Loadman *et al*., 1986; Katavic *et al*., 1989). During the initial stage of rearing, cannibalism is attributed by biting the abdomen or tail dominates, as the size homogeneity among the larvae is low (Baras & Jobling, 2002). When the size heterogeneity increases, cannibalism is mainly attributed by complete prey ingestion (Hecht & Pienaar, 1993; Biswas *et al*., 2018). The approaches made to increase the survival during seed rearing of striped murrel involves extrinsic factors such as reduced stocking density, routine size-grading, provision of shelters and dark condition (Kumar and Mohanty, 2018; Kumar *et al*., 2018). However, all these processes require time and also may cause extra stress to the fishes. The use of anti-aggressive in the diet might be an alternative way to improve survival during rearing of fry. Tryptophan is an essential amino acid for fish, which is a precursor of serotonin (5-HT) and is widely used to reduce aggressive behaviour (Winberg *et al*., 2001; Hseu *et al*., 2003; Höglund *et al*., 2005), increase stress-releasing effects (Johnston et al. 1990) and feeding performance (Pedro *et al*.,1998; Ortega *et al*., 2005). The dietary supplementation of tryptophan has been reported to reduce the cannibalism in the fry rearing of many fishes (Kumar *et al*., 2017; Biswas *et al*., 2018). Hence the effect of dietary L-tryptophan supplementation on growth, survival and cannibalism of *C. striatus* fry was evaluated in the present study.

MATERIALS & METHODS

Post larvae of *C. striatus* were procured from a private fish farm from Tamil Nadu, India. After proper acclimatization, the post larvae were fed with natural food like planktons up to 8 dph (days post hatching). Subsequently, weaning was done with a commercial diet (crude protein: 40% and crude lipid: 8%) up to 15 dph. The resulting post larvae were obtained and stocked for the experiment. The experiment was conducted for 45 days. The fish were reared in twelve FRP tanks of 100-L capacity (water volume 50-L) provided with continuous aeration throughout the study period. A group of 25 post larvae (0.19 ±0.001g) were stocked randomly in triplicates following a completely randomized design.

L-tryptophan was supplemented in the diet of fry at 0.6% (T_1), 1.2% (T_2) and 1.8% (T_3) and a control was maintained. The commercial fish larval diet was used to prepare the experimental diets. The experimental diet was prepared by sprinkle method as described by Krol and Zake (2016) and Biswas *et al.* (2018). The tryptophan (crystalline L-tryptophan, Himedia) for each treatment was weighed, dissolved in hot water plus ethanol (80%) and then sprinkled over the commercial diet. The diets were dried in oven at 37^0 C for 1 h and stored in a refrigerator at 4°C until use. The control diet was also sprinkled with same ethanol solution without adding tryptophan solution to avoid unpalatability of the diet.

The tanks were provided with seasoned ground water during the study period. All groups of fish were fed thrice a day *ad-libitum* under a normal light regime (12:12). Daily 50% of water exchange was done with chlorine-free seasoned water. The water quality parameters such as temperature, pH, dissolved oxygen (DO), free carbon dioxide (CO_2), alkalinity, hardness, ammonia-N, and nitrate-N were analysed every week following the standard method (APHA, 2005) and the values are presented in Table 1. Sampling was done once in a fortnight and the following parameters were recorded.

The growth response and survival were measured by using the following formulae:

Length increment (cm) = Final length - initial length

Body weight gain (BWG; %)= 100 ([Final mean body weight -Initial mean body weight] / initial weight)

Specific growth rate (SGR; % day^{-1})= 100 ({ln [Final mean body weight] -ln [Initial mean body weight]} /experimental period)

Mean daily weight gain (%) = 100 ([total final weight - total initial weight] / days of experiment)

Feed conversion ratio (FCR)= Total feed intake (dry weight)/Total live weight gain

Survival percentage was calculated at the end of the experiment by counting the number of fish in the tank and is calculated as follows:

Survival (%) =100 (Number of surviving fish / Total number of larvae stocked)

Cannibalism (%) = 100 × (Initial number of fish – [Final number of fish + Number of dead fish registered]) / Initial number of fish

The experimental data were analyzed using statistical package SPSS (Version 16.0, IBM, Chicago, Illinois, USA), in which data were subjected to one-way ANOVA and Duncan's multiple range tests to determine the significant differences among the means. A 5% probability (P<0.05) was chosen to determine the statistically significant difference among the treatments means. Results are presented as mean ± SE (standard error).

RESULTS

The growth performance of *C. striatus* fry under different dietary tryptophan treatments is given in Table 2. The final weight (1.54 ±0.01g), weight gain (699.70 ±8.10%), and specific growth rate (4.62 ±0.02) were found to be significantly higher in control group followed by T_1 whereas, significantly lower growth was found in T_3 treatment groups (P<0.05). However, significantly higher survival (85.33±2.67%) was observed in tryptophan supplemented groups with no significant difference among them (P>0.05). Meanwhile, the control group showed significantly lower survival (57.33 ±1.33%) among the treatments (P<0.05). Similarly, maximum cannibalism (31 ±1.33%) was observed in the control group (%), whereas no significant difference in cannibalism (%) was reported among the tryptophan supplemented groups as it is depicted in Fig. 1. The highest FCR (0.79 ±0.04) and lowest PER (3.19 ±0.17) values were recorded in control fed group, which was significantly different from the tryptophan supplemented groups.

TABLE 1. Physico-chemical parameters of water in different experimental groups

Parameters	Control	T1	T2	T3
Dissolved oxygen (mg l^{-1})	6.5-7.2	6.4-7.2	6.5-7.4	6.1-7.3
Temperature (^{o}C)	27.3-29.8	27.1-29.7	27.1-29.8	27.0-29.9
Water pH	7.5-7.8	7.3-7.6	7.4-7.6	7.2-7.5
Carbon dioxide (mg l^{-1})	1.1-1.9	1.2-1.9	1.1-.1.8	1-1.7
Total alkalinity (mg l^{-1})	45-61	48-65	44-68	45-65
Hardness (mg l^{-1})	35-41	33-39	34-43	34-42
Ammonia (mg l^{-1})	0.02-0.04	0.01-0.05	0.01-0.04	0.01-0.05

TABLE 2. Growth Parameters (mean ± SE) in different experimental groups fed with different level of tryptophan.

Parameters	Control	T1	T2	T3	P-value
Initial stock (nos.)	25	25	25	25	-
Initial weight (g)	0.19±0.001	0.19±0.001	0.19±0.001	0.19±0.001	0.922
Final weight (g)	1.54±0.01^d	1.49±0.01^c	1.21±0.01^b	1.05±0.02^a	0.000
Weight gain (%)	699.70±8.10^d	668.99±8.23^c	529.72±4.86^b	444.03±8.30^a	0.000
SGR (% day^{-1})	4.62±0.02^d	4.53±0.02^c	4.09±0.02^b	3.76±0.04^a	0.000
FCR	0.79±0.04^b	0.70±0.01^a	0.66±0.01^a	0.69±0.02^a	0.019
PER	3.19±0.17^a	3.58±0.02^b	3.80±0.05^b	3.60±0.10^b	0.016
Survival (%)	57.33±1.33^a	84.00±2.31^b	82.67±1.33^b	85.33±2.67^b	0.000

*Mean values with the same superscripts in each row are not significant (P>0.05). Values are means of three replicates of each experimental diet ± standard error (SE).

*SGR: Specific growth rate; FCR: Feed conversion ratio; PER: Protein efficiency ratio

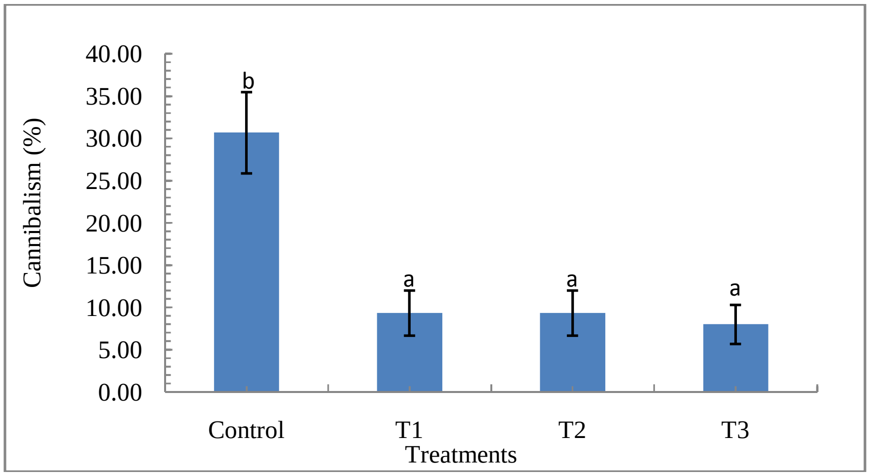

FIGURE 1. Effects of dietary tryptophan on cannibalism (%) of *Channa striatus* fry. Mean values are data of three replicates (n=3). Same letters on bars indicate no significant differences at (p>0.05) level.

DISCUSSION

The range of physico-chemical parameters of water in different experimental groups were within the acceptable limits as reported for striped murrel fry rearing (Kumar *et al.*, 2018) which indicated prevalence of appropriate growing environment for the species. In the present study, significantly higher growth was reported in control fed group when compared with tryptophan-supplemented diets. The lower growth performance in tryptophan-supplemented treatments might be due to the fact that the dietary supplementation of tryptophan increases brain 5-HT concentration, which in turn significantly reduces feed intake and hence growth (Pedro *et al*., 1998; Ortega *et al*., 2005; Biswas, 2019). Moreover, it has been reported that the 5-HT can directly act on somatotrophs in the pituitary gland and hinder the growth hormone secretion in goldfish (Peng and Peter, 1997). The growth hormone acts an important role in stimulating growth as well as food intake in fish (Lin *et al*., 2000; Peng and Peter, 1997). Similar observation was also reported in several other fish species, such as grouper (Hseu *et al*., 2003), European sea bass (Papoutsoglou *et al*., 2005) and pabda (Biswas *et al*., 2018). The study showed significantly higher growth performance in T_1 (0.6%) tryptophan supplemented group compared to higher tryptophan supplemented groups i.e. 1.2% (T_2) and 1.8% (T_3). Biswas *et al.* (2018) reported that much higher level of dietary tryptophan supplementation reduces the growth of pabda fry. They also reported that the tryptophan supplementation beyond 2% level decreases the growth performance without compromising the survival (%). Moreover, Hseu *et al.* (2003) also reported a reduction in growth performance in juvenile grouper (*Epinephelus coioides*), when supplemented dietary tryptophan level beyond 0.6% of the dry diet, which is relevant to the present finding. Hence, the decreased growth rate in higher tryptophan supplemented group is possibly due to higher brain serotonergic activity leading to reduce feed intake or appetite when supplemented beyond a level (Young 1996; Biswas *et al*., 2018). War and Haniffa (2011) and Kumar and Mohanty (2018) observed the cannibalistic mode in *C. striatus* during the early rearing stages. Cannibals were swimming vigorously, around the tank, ignoring live feed and targeting their own recessive siblings, resulting in heterogeneous growth among them. Cannibalism attributes a lot to mortality during nursery rearing, even where conditions appear to be ideal (Rawat *et al*., 2018), and it is more vigorous when the system have limited food. However, it also persists in predatory fish species even though the food resources are not limited (Hecht and Pienaar, 1993). In this study, tryptophan supplemented group showed significantly higher survival (%) of striped murrel fry than the control fed group. Although the striped murrel fries were fed at *ad-libitum* level, it still showed intense cannibalism in control fed groups than the tryptophan supplemented groups. Moreover, the control fed group also showed significantly higher cannibalism irrespective of feeding at *ad libitum* level. The higher survival and lower cannibalism in tryptophan fed group might be due the fact that the tryptophan supplementation has a calming effect on the aggression behaviour of striped murrel fry and consequently reduced the vigorous attacks to their own siblings. Control fed diet showed more aggressiveness and had fewer tendencies to escape from the dominants, which resulted in more cannibalism, as supported by the higher level of cannibalism percentage. The capability of tryptophan to repress aggression could be due to the increased activity of 5-HT in the brain which may suppress the aggressive behavior of dominants, as reported in several studies (Winberg *et al*., 2001; Hseu *et al*., 2003; Hoglund *et al*., 2005; Biswas *et al*., 2018). Moreover, Hoglund *et al.* (2007) revealed that L-tryptophan could reduce cannibalism and stress-induced anorexia in juvenile grouper, and Lepage *et al.* (2003) also found that it prevents stress-induced cortisol surge. Consequently, it can be concluded that increased levels of 5-HT due to tryptophan supplementation leads to a calming effect on dominant striped murrel fry and grew at a slower rate. Hence, subordinate fry might have obtained a better access to food and grows better in an optimum tryptophan supplementation level groups as reported in T_1 group in the present study. This is in agreement with the findings in other fishes such as *Oncorhynchus mykiss*

(Winberg *et al*., 2001), *Gadus morhua* (Hoglund et al., 2005), *Brycon amazonicus* (Wolkers *et al*., 2012), and *Lates calcarifer* (Kumar *et al*., 2017), where better survival was reported in tryptophan supplemented diet.

CONCLUSION

From the present study, it can be concluded that the growth of striped murrel fry is higher in the control fed group, although a significant reduction in survival was recorded. Moreover, for hatchery production of fishes, survivability is more important than the growth due to the economic returns. Hence supplementation of L-tryptophan at 0.6% level can be recommended to the farmers, as it reduces cannibalism without affecting the growth of striped murrel fry. Further studies are needed to undermine the effect of tryptophan supplementation on growth, survival and cannibalism at field level and also at the molecular level.

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