International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 3, Issue 2, pp: 239-244, 2018
UGC Approved Journal No. 46511
http://www.ijzab.com

Research Article

A COMPARATIVE STUDY OF GROWTH, METABOLISM AND DIGESTIVE ENZYME ACTIVITIES OF PINEALECTOMIZED AND NON-PINEALECTOMIZED CATFISH (*HETEROPNEUSTES FOSSILIS)*

Reeti Panchal, *Sudesh Rani, Poonam and Manju

Department of Zoology, Maharshi Dayanand University, Rohtak, Haryana-124001, India

Article History: Received 07th April 2018; Accepted 28th April 2018; Published 30th April 2018

ABSTRACT

A 60 days experiment was done to find out the importance of melatonin hormone dose on growth and metabolism of pinealectomized and non-pinealectomized catfish *Heteropneustes fossilis.* Experimental fish were fed with laboratory prepared formulated feed. All the experimental fish were having the mean body weight of 5-20 g was divided into two groups, pinealectomized and non-pinealectomized. Each group has two replicates. Melatonin injection of 25 and 50 mg were given to both the groups. Results of the present study indicated that Melatonin injected catfish of non-pinealectomized group shows maximum growth as compared to catfish received melatonin in pinealectomized group. The specific growth rate (SGR) and protein efficiency ratio (PER) values were higher in non-pinealectomized catfish as compared to pinealectomized catfish. However, the FCR valued were increased in melatonin received pinealectomized group as compared to melatonin administered non-pinealectomized group of catfish. The biometric indexes, hepatosomatic index (HSI) and viscerosomatic index (VSI) recorded maximum in pinealectomized fish as compared to non-pinealectomized catfish. Also, the digestive enzyme activities (protease, amylase, cellulase and lipase) were increased in melatonin administered non-pinealectomized catfish as compared melatonin injected pinealectomized catfish. The value for glycogen content in muscle was recorded higher in melatonin receiving pinealectomized fishes however the glycogen content in liver shows maximum values in melatonin receiving non-pinealectomized catfish. The results of this experiment showed that melatonin administered non-pinealectomized fish resulted in an increased growth, metabolism and digestive enzyme activities as compared to melatonin receiving pinealectomized catfish.

Keywords: Catfish, Growth, Melatonin, Non-pinealectomized, Pineal gland, Pinealectomized.

INTRODUCTION

Now a day biggest challenge is to feed billions of population in near future. The fisheries and aquaculture plays an important role according to (FAO, 2014) reports. Culture of catfish is important for export as well as domestic use. As far as this demand is conserved this also give employment to large no. of people (Krishnan, 1998). Latest techniques and equipments must come up, to fulfill our increasing need for aquaculture products which should be convenient and cheaper. *Heteropneustes fossilis*, commonly known as Singhi. It contains maximum protein and iron level due to this reason it is highly recommended food fish (Bhatt, 1968). Singhi shows a large number of characteristics like its large survival time in where oxygen is low (Haniffa & Sridhar, 2002; Saha & Ratha, 1998) compared with other fish species which makes it an important and valuable species for aquaculture practices. The aquaculture of *H. fossilis* require less production cost but its market value increasing day by day due to its high nutritional flesh quality (Alam *et al*.,1993). *Heteropneustes fossilis* is also capable to tolerate high salinity (Thakur, 1991), maximum fecundity (Radhakrishnan & Sugumaran, 2010), and can fed on supplement diets at rising temperature. Another

*Corresponding Author: Dr. Sudesh Rani, Assistant Professor, Department of Zoology, M.D. University, Rohtak-124001, Haryana, India. Email: sudesh_zoology@rediffmail.com, Mobile: +91 9729825140.

characteristic of this fish is that Singhi is hardy in nature and has the ability to survive in low ph levels.

In fish physiology pineal organ is very important as it is an important candidate of central nervous system. The pineal organ has transformed from a photo sensory organ into an endocrine gland, during vertebrate evolution (Collin, 1971). Melatonin hormone is released from the photo receptor cells of the pineal organ. Generally, the pineal organ is situated in the skull below a window through which light enters. The pineal organ attached by a slender stalk to the diencephalon roof and appears as a vesicle. The pineal organ gives information on duration of day length, spectral content and the intensity of the light. The pineal cells take up the essential amino acid tryptophan to synthesize the melatonin.

Serotonin is a monoamine chemical and neurotransmitter that is formed from tryptophan by two enzymatic steps. Tryptophan with the help of enzyme hydroxylase (TpOH) synthesizes hydroxytryptophan, and by tryptophan hydroxylation and the hydroxytryptophan is converted into serotonin by the aromatic amino acid decarboxylase. Studies showed that in continuous darkness (DD) an increased level of melatonin production takes place, whereas constant light (LL) suppresses the melatonin production. As fish is a poikilotherm animal hence it directly influenced by the temperature of the water. Many experimental studies have shown that through the regulation of the AANAT2 activity, temperature act directly on the pineal gland to modulate melatonin production (Benyassi *et al*., 2000; Falcon *et al*., 1999a; Falcon, 1999b). Endocrine glands by their hormonal release regulate the growth and metabolism processes and few of these hormones are regulated by the pineal organ. Hence pineal organ is very important as far as the growth is concerned. There are many studies which prove the role of pineal in growth of fish like in gold fish. The carbohydrate metabolism is controlled by altering insulin responsiveness by the pineal gland (Delahunty & Tomlinson, 1984). The disappearance in plasma glucose and reduction in glycogen amount in liver occurs by the pineal removal in *H. fossilis*. The total amount of lipid was affected in the liver of young pinealectomized sturgeon *Acipenser baeri*. In the Atlantic salmon (Salmo salar), (Porter *et al*., 1998) pineal gland and its hormone play an important role as intermediaries in the transfer of photic information to regulate daily time activities. Keeping importance of pineal gland on growth of fish a comparative study of growth, metabolism and digestive enzyme activities in pinealectomized and non-pinealectomized catfish *H. fossilis* was carried out.

MATERIAL AND METHODS

Experimental area

This experiment was carried out in the fisheries and animal behaviour laboratory, Department of Zoology, Maharshi Dayanand University, Rohtak, Haryana, India.

Experiment set up

The catfish, *Heteropneustes fossilis* was taken as experimental fish for the present study. Catfish with (mean body weight 5-20 g) were collected from Sultan aqua integrated education and research foundation V.P.O. Nilokheri Karnal, Haryana. Before start of the experiment the fishes were acclimatized under photoperiod (LD 12:12) and temperature ranges from 25 ± 1˚C under laboratory condition for 15 days. After than experiment was setup the fishes were divided into two groups pinealectomized and non-pinealectomized. Six aquariums were used under each group. This experiment was performed in replicate so twelve aquariums was used and fifteen fishes were placed in each aquarium.

The water of the aquarium was replenished with stored tap water daily. Initial length and weight of the fishes were recorded and then introduced in glass aquarium (60 × 30 × 30). The formulated feed was given to the fishes twice daily, in the morning (at 8: AM) and in the evening (at 5: PM) at the rate of 5% of their body weight. The experiment was carried out for 60 days.

Procedure of pinealectomy

The pineal removal is an important procedure of decreasing the melatonin level in night time. Pinealectomy (Px) of fishes was done by following procedure of (Rani and Sabhlok, 2014). During pinealectomy catfish wrapped in napkin and a sharp incision was made on the skin covers the pineal fontanelle to form a V shaped flap. The pineal fontanelle covering the membranous connective tissue was extracted. Then very carefully with the help of forceps the pineal stalk along with pineal vesicle was removed (Figures 1 & 2). The area from where pineal is removed was cleaned with 70% ethanol to avoid any infection in fishes. After pinealectomy the fishes were then returned aquarium water.

Melatonin

Melatonin solution was prepared by following the method of (Rani and Sabhlok, 2014). Melatonin (25, 50 mg) was dissolved in (2, 4 ml) of 100% ethanol and diluted with teleost saline (20 mg Na_2Co_3/100 ml of 0.6% NaCl). Every week the fresh solution of melatonin was made and stored in dark bottles. The melatonin injections were given to the fishes after 2 pm, three times in a week.

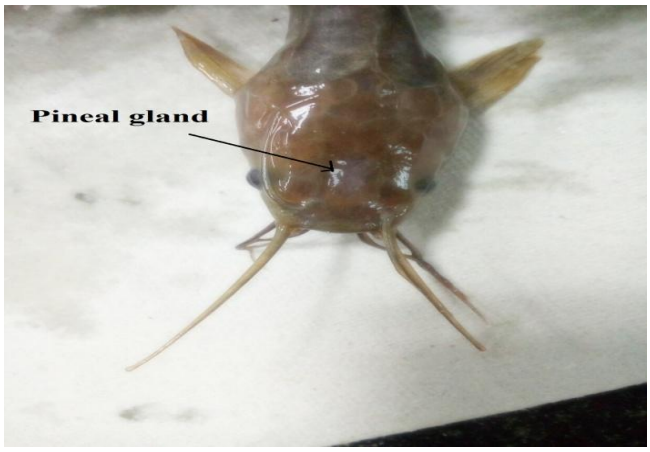

Figure 1. Photograph of the catfish *H. fossilis* shows pineal gland (Before pinealectomy).

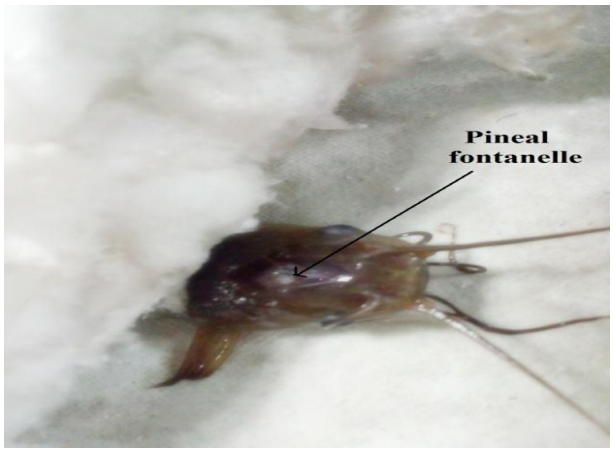

Figure 2. Photograph of the catfish *H. fossilis* shows pineal fontanelle (After pinealectomy).

Experimental procedure

Fishes were fasting while the injections were given. The I.P. more often tolerated by unanesthetized fish. Further this I.P. method is very useful for drugs that are not soluble in water or difficult to dissolve in water. In this procedure melatonin was given to the fishes using a 0.5 ml syringe close to the ventral midline posterior to the pelvic fins following the procedure of (De Vlaming, 1980). Dose pattern was following according to Aripin *et al.* (2014) with small manipulations (Figure 3).

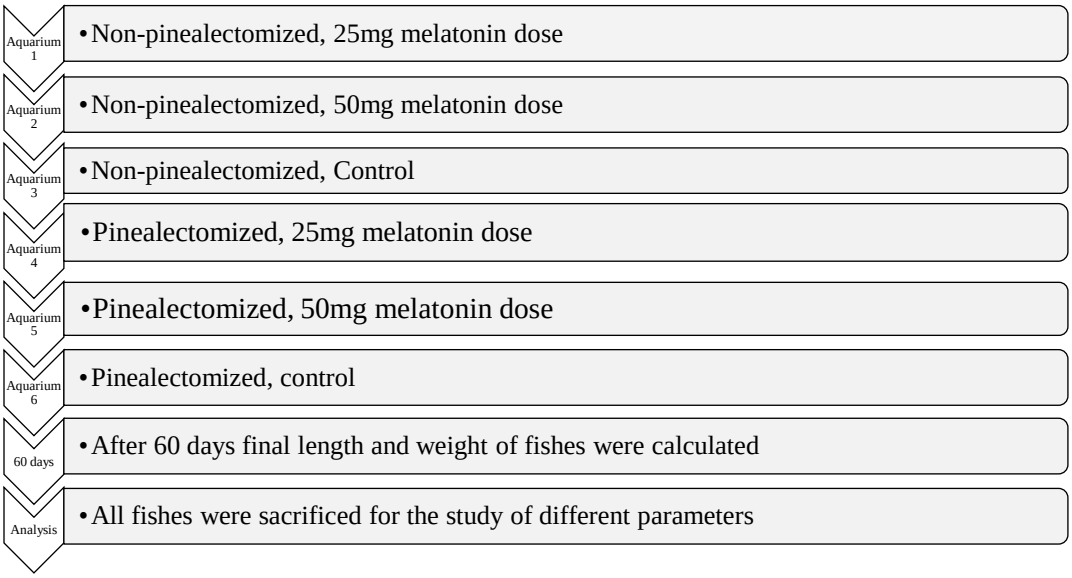

Figure 3. Melatonin dose pattern given *H. fossilis.*

Samples of liver, muscle and intestine were weighted and stored at -80˚C for further analysis

Parameters like digestibility, nutrient retention and digestive enzyme activity were calculated by Kunitz (1947), Bernified, (1955), Colowick & Kaplan, (1955), Sadasivam &Manickam (1996).

Glycogen content in Muscle and liver were calculated by following the procedure of (AOAC, 1995).

Biometric indexes were calculated by the formulae:

Hepatosomatic index (HSI) = Liver weight Hepatosomatic index (HSI) = Liver weight × 100/body weight and viscerosomatic index (VSI) = Visceral weight × 100/body weight.

Growth parameters were studied by following the procedure of (Sevier *et al.*, 2000).

Live weight gain (g) = $W_2 - W_1$

Where, W_2= final weight (g)
W_1= initial weight (g)

$$\text{Growth per cent gain in body weight} = \frac{W2 - W1}{W1 \times 100}$$

$$\text{Specific growth rate} = \frac{\ln W2 - \ln W1}{t \times 100}$$

$$\text{Feed conversion ratio} = \frac{\text{Feed given (dry wt. g)}}{\text{Body weight gain (wet wt. g)}}$$

$$\text{Protein efficiency ratio} = \frac{W2 - W1}{\text{Feed} \times \text{feed protein}}$$

Where, feed = total feed given

$$\text{Hepato} - \text{somatic index} = \frac{\text{Liver weight (g)} \times 100}{\text{Body weight}}$$

$$\text{Viscero} - \text{somatic index} = \frac{W2 - W1}{\text{Body weight}}$$

Statistical analysis

The one-way analysis of variance (ANOVA) followed by Turkey HSD test was applied to find out the significance of growth parameters and digestive enzyme activities in different groups.

RESULTS AND DISCUSSION

After 60 days of the experiment, the growth performance and digestive enzyme activity of *Heteropneustes fossilis* were recorded (Table 1 and 2). The results of our study indicated that melatonin treatment suppressed the weight of fish. The digestive enzyme activities and glycogen content in liver were decreased in melatonin administered pinealectomized catfishes as compared to melatonin administered non pinealectomized catfishes. The values of SGR and FCR were reduced in melatonin administered non-pinealectomized fishes as compared to melatonin received pinealectomized fishes. Whereas growth % gain and PER values were decreased in melatonin received pinealectomized as compared to melatonin treated non-pinealectomized catfishes. However the pinealectomized fishes received melatonin injections showed maximum value of glycogen content in muscle as compared to non-pinealectomized fishes. The biometric indexes, like viscerosomatic index (VSI) and hepatosomatic index (HSI) recorded maximum values in pinealectominzed fishes as compared to non - pinealectomized fishes. Melatonin administration decrease growth rate and body weight in trout *Oncorhynchus mykiss* (Taylor *et al*.,2005). These results are in Favour to our results as in our finding same pattern observed. Whereas (Aripin *et al*., 2015) recorded that melatonin did not affect the weight gain in *Clarias macrocephalus* male. When different concentrations of melatonin were providing in vitro, the high level of growth hormone was released by cultured trout pituitary cells or glands (Falcon *et al*., 2003). The fish grow differently depending on the circadian time feeding. Hence, the growth of fish follows seasonal pattern of different day length. Melatonin also induced inhibition of prolectin production, under conditions which stimulate growth hormone production (Falcon *et al*., 2003). Growth hormone and prolectin hormones act in an antagonistic manner (Nguyen *et al*., 2008). Singh *et al*. (2012) indicated in their findings on Nile tilapia *Oreochromis niloticus*, that exogenous melatonin administration (25µg/L for 21 days) showed a 36.6% decrease in SGR% per day as compared to untreated group. The melatonin dose 10µg/g body weight in gold fish *Carassius auratus* decrease weight (Lopez *et al*., 2006). Melatonin administration in gold fish decreased specific growth rate as well as body weight (Pedro *et al*., 2008), in our study same pattern were recorded as SGR% also reduced in melatonin injected fish pinealectomized as well as non-pinealectomized.

As digestive enzyme activities (specific protease, amylase, cellulase as well as lipase were reduced in fish pinealectomized or non-poinealectomized which received high dose 50 mg of injection. These findings are synonyms to the findings of (Rani & Sabhlok, 2014) where these parameters follow the same pattern as that of ours. Very recently (Zhang *et al*., 2018) recorded that melatonin improve the digestive enzyme activity in *Chinese mittan* crab.

Table 1. Effect of low and high doses of melatonin on growth performance feed conversion ratio, and protein efficiency ratio of pinealectomized and non pinealectomized catfish *Heteropneustes fossilis* under laboratory conditions.

Parameters	1(NP) 25 mg (M)	2(NP) 50 mg (M)	3(NP)Control	4(P) 25 mg (M)	5(P) 50 mg (M)	6(P) Control
Initial weight	7.91	8.62	8.05	8.64	8.41	8.07
Final weight	7.78	7.86	8.36	7.78	8.1	7.95
Live weight gain	-0.13	-0.76	0.31	-0.86	-0.31	-0.12
Growth % Gain	-1.64 ± 0.79	-8.82 ± 4.82	3.85 ± 0.05	-9.95 ± 0.43	-3.69 ± 0.62	-1.49 ± 1.14
Specific growth rate	-0.03 ± 0.01	-0.15 ± 0.09	0.06 ± 0.00	-0.17 ± 0.01	-0.06 ± 0.01	-0.02 ± 1.40
Feed conversion ratio	3.740 ± 0.014	3.798± 0.080	3.678±0.020	3.970± 0.010	3.797 ± 0.030	3.679±0.050
Protein efficiency ratio	-0.31 ± 0.16	-1.78 ± 1.03	0.70 ± 0.01	-1.95 ± 0.06	-0.70 ± 0.13	-0.29 ± 0.22
Hepato-somatic index	0.65 ± 0.138	0.92 ± 0.056	1.12 ± 0.063	0.79 ± 0.003	0.83 ± 0.110	1.02 ± 0.300
Viscero-somatic index	1.72 ± 0.14	2.65 ± 0.23	2.24 ± 0.00	1.91 ± 0.01	1.98 ± 0.23	3.26 ± 0.39

All the values are mean ± S.E. of mean. (NP), Non-pinealectomized, (P) pinealectomized, (M) melatonin dose.

Table 2. Effect of the low and high doses melatonin specific protease amylase, cellulase, lipase, muscle glycogen and liver glycogen pinealectomized and non pinealectomized catfish *Heteropneustes fossilis* under laboratory conditions.

Parameters	1(NP) 25mg (M)	2(NP) 50mg (M)	3(NP) control	4(P) 25mg (M)	5(P) 50mg (M)	6(P) Control
Specific protease activity	1.63 ± 0.02	1.49 ± 0.12	1.72 ± 0.01	1.24 ± 0.09	1.07 ± 0.01	0.67 ± 0.10
Specific amylase activity	1.67 ± 0.34	1.50 ± 0.17	1.70 ± 0.20	1.29 ± 0.04	0.74 ± 0.07	0.60 ± 0.03
Specific cellulase activity	0.94 ± 0.04	0.74 ± 0.03	0.96 ± 0.05	0.65 ± 0.01	0.51 ± 0.03	0.48 ± 0.02
Specific lipase activity	1.60 ± 0.11	1.27 ± 0.04	1.78 ± 0.01	1.16 ± 0.14	1.06 ± 0.03	0.83 ± 0.11
Muscle glycogen	1.87 ± 0.07	2.28 ± 0.08	1.43 ± 0.12	2.69 ± 0.06	3.27 ± 0.24	3.74 ± 0.01
Liver glycogen	5.06 ± 0.33	4.62 ± 0.20	6.54 ± 0.52	3.62 ± 0.17	2.52 ± 0.07	1.68 ± 0.06

All the values are mean ± S.E. of mean. (NP), Non - pinealectomized, (P) pinealectomized, (M) melatonin dose.

CONCLUSION

It is widely accepted that pineal gland releases melatonin hormone which regulate a large number of physiological processes of body. The amount of induced hormones which are necessary for the growth may be increased when the production of the melatonin hormone is decreased by the use of some chemicals. The melatonin administration affects many physiological processes and when the pineal gland is removed surgically it also affect various body functions. The results of this experiment showed that the melatonin administration and pinealectomy suppressed the growth performance and digestive enzyme activity in melatonin treated pinealectomized catfishes as compared to melatonin received non-pinealectomized catfish, *Heteropneustes fossilis.*

REFERENCES

Alam, M. S., Jahan, M., Hossain, M. M., & Islam, M. S. (2009). Population genetic structure of three major river populations of rohu, Labeo rohita (Cyprinidae: Cypriniformes) using microsatellite DNA markers. *Genes & Genomics*, *31*(1), 43-51.

Alok, D., Krishnan, T., Talwar, G., & Garg, L. C. (1993). Induced spawning of catfish, Heteropneustes fossilis (Bloch), using D-Lys6 salmon gonadotropin-releasing hormone analog. *Aquaculture*, *115*(1-2), 159-167.

Aripin, S.A., Jintasataporn, O., & Yoonpundh, R. Effects of Melatonin on Clarias Macrocephalus Female Broodstock Performance. *International Journal of Sciences: Basic and Applied Research 21*(1), 129-137.

AOAC, 1995. Official methods of analysis of the Association of Official Analytical Chemist. 15th eds., Washington, D.C., 1 and 2, 1298.

Benyassi, A., Schwartz, C., Coon, S. L., Klein, D.C., & Falcon, J. (2000). Melatonin synthesis: arylalkylamine N-acetyltransferases in trout retina and pineal organ are different. *Neuroreport*, *11*(2), 255-258.

Bernified, P., 1955. In: Methods of enzymology. *Academic Press, New York*, 1, 149.

Bhatt, V. (1968). Studies on the biology of some freshwater fishes. Part VII. *Heteropneustes fossilis*, 99-115.

Collin, J.P. (1971). Differentiation and regression of the cells of the sensory line in the epiphysis cerebri. *The pineal gland*, 79-125.

Colowick, S.P. and Kaplan, N.O., 1955. Methods of enzymology. *Academic Press, New York.*, 1, 627

Coon, S. L., Begay, V., Deurloo, D., Falcon, J., & Klein, D.C. (1999). Two arylalkylamine N-acetyltransferase genes mediate melatonin synthesis in fish. *Journal of Biological Chemistry*, *274*(13), 9076-9082.

De Pedro, N., Martínez Alvarez, R. M., & Delgado, M. (2008). Melatonin reduces body weight in goldfish (Carassius auratus): effects on metabolic resources and some feeding regulators. *Journal of pineal research*, *45*(1), 32-39.

Delahunty, G., & Tomlinson, M. (1984). Hypoglycemic effects of melatonin in the goldfish, Carassius auratus. *Comparative Biochemistry and Physiology Part A: Physiology*, *78*(4), 871-875.

Falcon, J. (1999). Cellular circadian clocks in the pineal. *Progress in neurobiology*, *58*(2), 121-162.

Falcon, J., Gothilf, Y., Coon, S., Boeuf, G., & Klein, D. (2003). Genetic, temporal and developmental differences between melatonin rhythm generating systems in the teleost fish pineal organ and retina. *Journal of Neuroendocrinology*, *15*(4), 378-382.

FAO, & WFP (2014). The state of food insecurity in the world, 80.

Kather Haniffa, M. A., & Sridhar, S. (2002). Induced spawning of spotted murrel (Channa punctatus) and catfish (Heteropneustes fossilis) using human chorionic gonadotropin and synthetic hormone (ovaprim). *Veterinarski arhiv*, *72*(1), 51-56.

Kunitz, M., 1947. Crystalline soybean trypsin inhibitor: 11. General properties. *Journal of Genentics and Physiology*, 30, 291-310.

Krishnan, L. (1998). Status of seed production and culture of brackish water fin fishes in India. *Techological Advancement in Fisheries*, 29-36.

Lopez Olmeda, J., Madrid, J., & Sanchez Vazquez, F. (2006). Melatonin effects on food intake and activity rhythms in two fish species with different activity patterns: diurnal (goldfish) and nocturnal (tench). *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, *144*(2), 180-187.

Nguyen, N., Stellwag, E. J., & Zhu, Y. (2008). Prolactin-dependent modulation of organogenesis in the vertebrate: recent discoveries in zebrafish. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *148*(4), 370-380.

Porter, M., Randall, C., Bromage, N., & Thorpe, J. (1998). The role of melatonin and the pineal gland on development and smoltification of Atlantic salmon (Salmo salar) parr. *Aquaculture. 168*(1-4), 139-155.

Radhakrishnan, M., & Sugumaran, E. (2010). Effect of Sugarcane Bagasse and Supplemental Feed on Certain Reproductive Characterestics of the Catfish Heteropneustes fossilis (Bloch.). *Journal of Fisheries International*, *5*(4), 58-60.

Rani, S. and Sabhlok, V.P., 2014. Effect of melatonin administration on growth and metabolism of catfish *Clarias batrachus* under different photoperiods. *Journal of International Academic Research for Multidisciplinary*, *2*, 627-635.

Sadasivam, S. and Manickam, A., 1996. Biochemical methods. New Age International (P) Ltd. Publishers. 126-128.

Saha, N., & Ratha, B. (1998). Ureogenesis in Indian air-breathing teleosts: adaptation to environmental constraints. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *120*(2), 195-208.

Sevier, H., Raae, A.J. and Lied, E., 2000. Growth and protein turnover in (Atlantic samo salar L.) the effect of dietary protein level and protein particle size. *Aquaculture*. 185, 101-120.

Singh, R., Singh, A., & Tripathi, M. (2012). Melatonin induced changes in specific growth rate, gonadal maturity, lipid and protein production in Nile tilapia Oreochromis niloticus (Linnaeus 1758). *Asian-Australasian Journal of Animal Sciences*, *25*(1), 37.

Taylor, J., Migaud, H., Porter, M., & Bromage, N. (2005). Photoperiod influences growth rate and plasma insulin-like growth factor - I levels in juvenile rainbow trout, Oncorhynchus mykiss. *General and Comparative Endocrinology*, *142*(1-2), 169-185.

Thakur, N. K. (1991). Possibilities and problems of catfish culture in India *Journal Inland Fish Society of India*, *23*(2), 80-90.

Zhang, C., Yang, X.Z., Xu, M.J., Huang, G.Y., Zhang, Q., Cheng, Y.X., Ren, H.Y. (2018). Melatonin Promotes Cheliped Regeneration, Digestive Enzyme Function, and Immunity Following Autotomy in the Chinese Mitten Crab, Eriocheir sinensis. *Frontiers in Physiology*, *9*, 269.