ASIAN J. EXP. BIOL. SCI.VOL 4(4) 2013: 561-566

ISSN 0975-5845
AJEBS
http://www.ajebs.com

ORIGINAL ARTICLE

Histological Changes in the Intestine of *Channa striatus* Grow-outs Fed With Different Fat Sources: Effects of Dietary Manipulation

R. Dayal[1], P. P. Srivastava[1,2,*], W. S. Lakra[1,3], A. Bhatnagar[4], S. Chowdhary[1], A. K. Yadav[5] and S. M. Srivastava[1]

[1]National Bureau of Fish Genetic Resources, Canal Ring Road, Teli Bagh, Lucknow – 227 002, UP, India
[2]Fish Nutition, Biochemistry and Physiology Division, Central Institute of Fisheries Education, Mumbai-400 061, MS, India
[3]Central Institute of Fisheries Education, Panch Marg, Off Yari Road, Mumbai-400 061, MS, India
[4]Department of Zoology, Kurukshetra University, Kurukshetra - 136 119, Haryana, India
[5]Aquaculture Research Training Unit,National Bureau of Fish Genetic Resources, Faizabad Road, Lucknow-226 105, India

Received 08-08-2013 **Revised 03-10-2013** **Accepted 20-10-2013**

ABSTRACT

In the present study the intestinal sac method (ex vivo) was used to evaluate the interactions between different fats in the diets and the histology of the gastrointestinal (GI) tract of Saul (Channa striatus). Saul was fed with six diets (L3HUF, F1; H3HUF, F2; MUSOL, F3; LINOL, F4; MIXOL, F5; SATOL, F6) and a control (NATFO, F7) with natural food. F1, contains 0.5% ω-3 fatty acid and 7.5% saturated oil; F2, contains 1.0% ω-3 fatty acid and 7.0% saturated oil; F3, contains 8.0% mustard oil; F4, contains 8.0% linseed oil; F5, contains 4.0% mustard oil and 4% linseed oil; F6, contains 8% saturated oil. Control and fishes fed with different fat had almost similar cellular appearance to intact intestinal mucosal epithelium, with no signs of prominent cellular damage. However, fishes fed with mustard oil in the diet depicting spaces at the base of the villi; fishes fed with linseed oil showing appearance of spaces between circular muscles and base of villi; fishes fed with mixed oil showing elongated lumen in villi, circular muscles are enlarged and fishes fed with saturated oil showing elongated lumen in villi. The results suggests that supplementation of different fat and/or essential fatty acids has direct relation with the histological changes in the intestinal tissue and observations conceptualize that dietary interventions of lipids in the fish feed has relation with cellular and tissue level modifications in the intestine.

Key words: *Channa striatus . dietary fats . histology . intestine.*

INTRODUCTION

Quality food and/ or feed in fish production system is essential to economically produce healthy, high quality and better fish production. Riche and Garling [1] demonstrated that fish cultured in intensive systems requires all nutrients in a complete pelleted diet since natural food is limited and fish cannot forage freely for natural livefoods. The study for the alteration in fish intestine has been elucidated to see the changes, if any, in the tissue on feeding the fish for a longer time on alternative feeds. Due to the constantly increasing world production of fish and other aquatic organisms, it is necessary to replace fish oil in diets with less expensive raw materials of plant origin. Therefore, monitoring histological structure of fish intestine is the method of choice in assessing the effects of nutrient mixtures that use raw materials of plant and/or animal origin, specially dietary fats of different origin. Histological analysis of the digestive system is considered a good and immediate indicator of the nutritional status of fish [2-4]. The intestine is the most important organs in digestion and absorption of nutrients from food, and therefore monitoring of these organs is considered necessary [5,6] and assessing the effects of ingredients used as raw materials of animal/ plant origin

particularly for different dietary fat contents.

The aim of the aquaculture feed production is the replacement of fish oil with less expensive source of fats, usually of plant origin. A low level of fibres, carbohydrates and indigestible anti-nutrients, high protein level, good amino acid profile, high digestibility and palatability are appropriate characteristics of good plant ingredients in fish feed [7,8]. Hardy [9] argues that the industry will soon run out of sufficient quantities of fish oil. Even if all these features are present in plant, it does not mean that it can be used as the complete replacement of fish oil. It is common that only a part of the oil in feed is replaced, but even such low level of replacement can have an effect on fish organism, primarily on the digestive tract. Recent research is pointing out possibilities of almost cent percent replacement of FM and fish oil in diets for carnivorous fish species like Atlantic salmon and Atlantic cod with proteins and fats of alternative origin without adverse effects on growth [10,11]. The inclusion of dietary sources of plant origin fat may imbalance the fatty acid and affecting cellular changes.

Thus, the interest of present study is to look into the effects of various dietary sources of fats in the diet of air- breathing fish, *Channa striatus*, locally known as saul, is a fish of great demand and attracts the attention of farmers for its high market value with medicinal properties. Since knowledge of the structure of the intestine is essential to the understanding of physiological and also normal/abnormal conditions on feeding diets manipulated with different dietary fats, this research was undertaken to describe the intestinal histology of *Channa striatus.* This experiment was carried to study the effects of dietary fats in the histological changes in the intestine of *Channa striatus* grow-outs.

MATERIAL S AND METHODS

Six diets (L3HUF, F1; H3HUF, F2; MUSOL, F3; LINOL, F4; MIXOL, F5; SATOL, F6) and a control (NATFO, F7) with natural food. F1, contains 0.5% ω-3 fatty acid and 7.5% saturated oil; F2, contains 1.0% ω-3 fatty acid and 7.0% saturated oil; F3, contains 8.0% mustard oil; F4, contains 8.0% linseed oil; F5, contains 4.0% mustard oil and 4% linseed oil; F6, contains 8% saturated oils. (Table 1). In order to evaluate the effect of different oil sources on the Intestine of *C. striatus*, the experiment was conducted in indoor condition in 14 (7 types of feed, 2 replicates) round plastic pools 300 litre capacity filled-up two-thirds with tube well water and covered with plastic covers . Each having two replications, stocked with 20 grow-out having an initial average weight 27.36$\pm$ 0.09g to 32.54 $\pm$ 0.41g were plotted in each of the plastic pool after proper acclimatization. The tanks were provided aeration from a portable aerator round the clock. During the experiment, the fishes were fed twice a day at 10:00 and 17:00 hours ad libitum per day. Rearing pools were cleaned every second day and about half of the water was replaced with fresh bore-well water to reduce the nitrogenous waste accumulated as debris and faecal matters.

After 12-weeks of feeding trials with seven feed combinations (Table-1) the animals were sacrificed. The distal intestine from control(F7, NATFO) and experimental fishes (feed with different fats (F1 to F6) were excised and fixed in 4 % formaldehyde and processed by standard histological techniques ie., kept in aqueous Bouin's fluid for 24-hr and washed for 8-hr in running tap water. The organs were routinely processed (dehydrated in ethanol series, embedded in paraffin, serially sectioned at 6μ). Sections of the intestine were stained with Haematoxylin and Eosin (HE) [12]. Histological slides were observed under microscope (Labomed, Model : Digi 2).

Table: 1 Ingredients composition (w/w) of feeds for *Channa striatus*.

Feed Ingredients	F-1 (L3HUF)	F-2 (H3HUF)	F-3 (MUSOL)	F-4 (LINOL)	F-5 (MIXOL)	F-6 (SATOL)	F-7 (NATFO)
Soybean meal	41.0	41.0	41.0	41.0	41.0	41.0	-
Starch Soluble	25.0	25.0	25.0	25.0	25.0	25.0	-
Casein	20.0	20.0	20.0	20.0	20.0	20.0	-
Carboxy Methyl Cellulose	2.0	2.0	2.0	2.0	2.0	2.0	-
Papain	0.5	0.5	0.5	0.5	0.5	0.5	-
Vitamin & Mineral Mix.	3.5	3.5	3.5	3.5	3.5	3.5	-
Omega – 3 HUFA	0.5	1.0	-	-	-	-	-
Saturated Oil	7.5	7.0	-	-	-	8.0	-
Mustard Oil	-	-	8.0	-	4.0	-	-
Linseed Oil	-	-	-	8.0	4.0	-	-
Live Fish/ Natural Food	-	-	-	-	-	-	100.0

L3HUF = Low Omega – 3 HUFA; H3HUF = High Omega – 3 HUFA; MUSOL = Mustard Oil; LINOL = Linseed Oil; MIXOL = Mixed Oil (Mustard Oil : Linseed Oil :: 1 : 1 w/w) ; SATOL = Saturated Oil; NATFO = Natural Food

RESULTS

The feed used in the present study did not have shown a marked changes and impact on histological alterations in the intestine of *Channa striatus* fed with Natural food (NATFO, F7) showing normal architecture of intestine with circular muscles, longitudinal muscles, serosa and villi (Fig-1). Intestine of *Channa striatus* fed with low quantity of highly unsaturated fatty acid (L3HUF) (F1) showing normal appearance of circular muscles, longitudinal muscles, serosa and villi (Fig-2). Intestine of *Channa striatus* fed with high quantity of highly unsaturated fatty acid (H3HUF) (F2) showing normal appearance of circular muscles, longitudinal muscles, serosa and villi (Fig-3). Intestine of *Channa striatus* fed with mustard oil in the diet (MUSOL) (F3) depicting normal appearance of circular muscles, longitudinal muscles, serosa and villi. More spaces at the base of the villi (Fig-4).

Intestine of *Channa striatus* fed with linseed oil (LINOL) (F4) showing appearance of spaces between circular muscles and base of villi. Normal circular muscles, longitudinal muscles and serosa are seen (Fig-5). Intestine of *Channa striatus* fed with mixed oil (MIXOL) (F5) showing elongated lumen in villi. Circular muscles are enlarged, longitudinal muscles and serosa are normally seen (Fig-6). Intestine of *Channa striatus* fed with saturated oil (SATOL) (F6). Showing elongated lumen in villi. Circular muscles less seen, longitudinal muscles are seen normally and serosa layer is reduced showing some detachment of base of villi observed with more spaces (Fig-7).

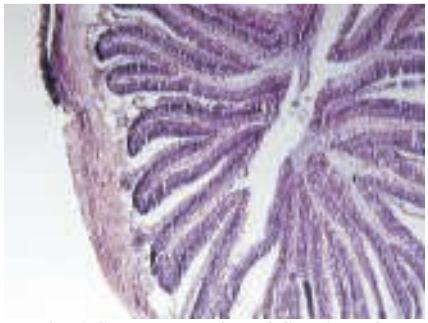

Fig.-1 Control Intestine of *C. striatus* fed with natural food (F7).

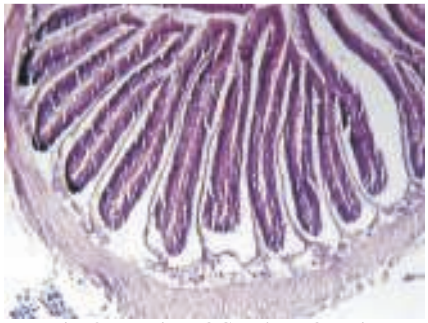

Fig.-2 Intestine of *C. striatus* fed with *L3HUF (F-1).*

Fig.-3 Intestine of *C. striatus* fed with *H3HUF (F-2).*

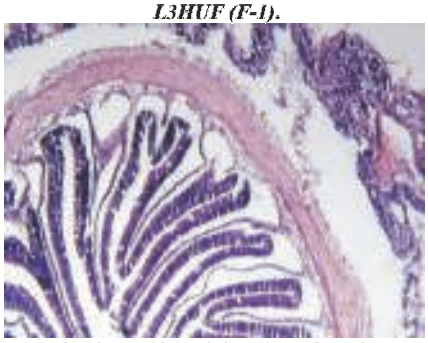

Fig.-4 Intestine of *C. striatus* fed with MUSOL *(F-3).*

Fig.– 1 Intestine of *C. striatus* fed with Natural feed (NATFO, F7) showing normal architecture of intestine with circular muscles, longitudinal muscles, serosa and villi. H/E X 125.

Fig. - 2 Intestine of *C. striatus* fed with low quantity of highly unsaturated fatty acid (L3HUF) (F1) showing normal appearance of circular muscles, longitudinal muscles, serosa and villi. H/E X 125.

Fig. - 3 Intestine of *C. striatus* fed with high quantity of highly unsaturated fatty acid (H3HUF) (F2) showing normal appearance of circular muscles, longitudinal muscles, serosa and villi. H/E X 125.

Fig. - 4 Intestine of *C. striatus* fed with mustard oil in the diet (MUSOL)(F3) depicting normal appearance of circular muscles, longitudinal muscles, serosa and villi. More spaces at the base of the villi. H/E X 125.

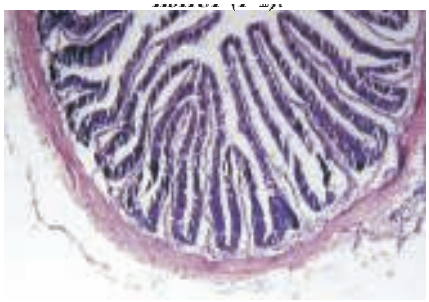

Fig.-5 Control Intestine of *C. striatus* fed with LINOL (F4).

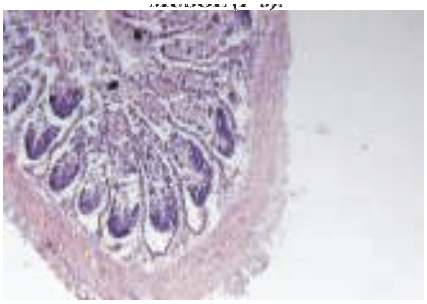

Fig.-6 Intestine of *C. striatus* fed with *MIXOL (F-5)*.

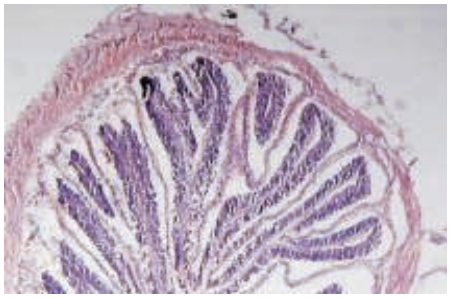

Fig.-7 Intestine of *C. striatus* fed with *SATOL (F-6)*.

Fig. - 5 Intestine of *C. striatus* fed with linseed oil (LINOL) (F4) showing appearance of spaces between circular muscles and base of villi. Normal circular muscles, longitudinal muscles and serosa are seen. H/E X 125.

Fig.- 6 Intestine of *C. striatus* fed with mixed oil (MIXOL) (F5) showing elongated lumen in villi. Circular muscles are enlarged, longitudinal muscles and serosa are Normally seen. H/E X 125.

Fig. - 7 Intestine of *C. striatus* fed with saturated oil (SATOL) (F6) showing elongated lumen in villi. Circular muscles less seen, longitudinal muscles are seen normally and serosa layer is reduced showing some detachment of base of villi observed with more spaces. H/E X 125.

DISCUSSION

The intestine is first organ affected by the dietary fat and its types, fatty acid profile etc., therefore, the impact of dietary fat on the histological architect has been studied in the grow-outs of *Channa striatus*. In the present study the impacts of diets (F1-F6, rich with different fats) on histology of the intestine were investigated. Histological structure of this part of the digestive system is: mucosa, which consists of the lamina epithelialis (simple columnar and glandular epithelium) and lamina propria (connective tissue); submucosae, consisting of two layers (stratum compactum and stratum granulosum); muscular layer and serosa [13]. This histological structure is not altered even in experiments in which fishes were fed with food containing heavy metals [14,15]. Histopathological changes in the intestine may vary depending on the species and feed used in the experiments. There seems to be no information on the deleterious effects of dietary protein on the histological structure of intestine. Flexibility of the piscine gastrointestinal tract to adjust to food availability is well illustrated; e.g. Atlantic cod with the high feed intake had relatively higher weight of different sections of the gastrointestinal tract compared to cod with the lower feed intake [16]. Gomez-Requeni et al. [17], where weight was depressed up to 30% in the group that had 100% replacement of FM with plant proteins and various oils. Hansen et al. [11] found a significant decline in feed intake with complete replacement of FM with plant ingredients in the diet for cod. Reduced appetite may be a consequence of replacement of fishmeal and fish oil by proteins of plant origin [18]. The finding[19] indicates that animal protein and plant lipids rich feeds with glucosamine were much acceptable than natural feeds for Asian catfish, *Clarias batrachus*. Uran et al. [20] reported that carp show signs of enteritis when fed high levels of soy oil in the diet. The findings[20] demonstrate the corroborative results of our findings.

In the present study, histological changes in the intestine of *Channa striatus* have been observed on feeding different fats, proliferation of villi, and serosa, mucosa and sub-mucosa as well as space in villi. The intestinal wall of *C. striatus* comprised of four distinct layers, viz. mucosa, submucosa, muscularis and serosa. Intestine of *C. striatus* fed

with Natural feed (NATFO, F7), (L3HUF) (F1) and (H3HUF) (F2) showing normal architecture of intestine. Intestine of *C. striatus* fed with mustard oil in the diet (MUSOL)(F3) depicting more spaces at the base of the villi. Intestine of *C. striatus* fed with linseed oil (LINOL) (F4) showing appearance of spaces between circular muscles and base of villi. Fish fed with mixed oil (MIXOL) (F5) showing elongated lumen in villi and Circular muscles are enlarged. Intestine of *C. striatus* fed with saturated oil (SATOL) (F6) showing elongated lumen in villi. However, showing some detachment of base of villi observed with more spaces. Similar finding have been reported by few authors [21-23]. Observations made by earlier workers relating to histopathological changes in intestine in response to various fat and protein are being enumerated here. The histological changes in intestine can also reduce growth performance on feeding different types of feed [24-27]. The proliferation, necrosis of serora and mucosa and rupture of villi have been reported by Konar [28] in *L. rohita*; Wong et al. [29] in *C. carpio* and *Ctenopharyngodon idellus*; Sastri and Gupta [30,31] in *C. punctatus*; Kumar and Pant[22] in *Barbus conchonius;* Bakthavathsalam et al. [32] in *C. punctatus* against exposure to different chemicals.

It is observed that *C. striatus* is technically prone to dietary intervention or manipulations in their nutrients composition including fats. The reason may be obvious because this fish is carnivore/ piscivore and mainly consume protein and fat rich diets of animal origin. And change in diet rich in fats have, therefore, impacted on cellular/ tissue levels changes in the intestine. Therefore, these dietary oils may be used in fish diet in combination with most acceptable fish oil or in solo in low quantity to reduce feed cost without affecting growth of grow-outs of *C. striatus* in culture system.

CONCLUSION

The observations in the present study conceptualize that dietary interventions of lipids in the feed has relation with cellular and tissue level modifications in the intestine *Channa striatus*.

ACKNOWLEDGEMENTS

Authors are grateful to the Director of the NBFGR, Lucknow for providing facilities to conduct the research work.

REFERENCES

[1]. Riche, M. and Garling, D. (2003) Feeding Tilapia in intensive re-circulatory systems. North Central Regional Aquaculture Centre and United State Department of Agriculture USDA. 1-4pp.

[2]. Hall, K.C. and Bellwood, D.R.(1995). Histological effects of cyanide, stress and starvation on the intestinal mucosa of *Pomacentrus coelestis*, a marine aquarium fish species. *Journal of Fish Biology* 47: 438-454.

[3]. Green, B.S. and McCornick, M.I. (1999). Influence of larval feeding history on the body condition of *Amphiprion melanopus*. *Journal of Fish Biology* 55:1273-1289.

[4]. Caballero, M.J., Izquierdo, M.S., Kjørsvik, E., Montero, D., Socorro, J., Fernández, A.J. and Rosenlund, G. (2003). Morphological aspects of intestinal cells from gilthead seabream (*Sparus aurata*) fed diets containing different lipid sources *Aquaculture* 225: 325-340.

[5]. Takashima, F. and Hibiya, T. (1982). An atlas of fish histology: normal and pathological features. Kodansha, distributed by Fischer, G., Tokyo.

[6]. Roberts, R. J. (1989). In: Fish Pathology. Baillière Tindall, London.

[7]. Francis, G., Makkar, H.P.S. and Becker, K. (2001). Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture* 199:197-227.

[8]. Marković, Z., Poleksić, V., Dulić, Z. and Stanković, M. (2009).Carp production intensification in traditional semi-intensive culture systems by application of extruded feed and selected fish fry. Aquaculture Europe, August 14-17, Trondheim, Norway, Book of Abstracts, pp. 498-499.

[9]. Hardy, R.W. (2010). Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. *Aquaculture Research* 41(5): 770-776.

[10]. Espe, M., Lemme, A., Petri, A, and El-Mowafi, A. (2007). Assessment of lysine requirement for maximal protein accretion in Atlantic salmon using plant protein diets. *Aquaculture* 263: 168-178.

[11]. Hansen, A.C., Rosenlund, G., Karlsen, O., Koppe, W. and Hemre, G. I. (2007). Total replacement of fish meal with plant proteins in diets for Atlantic cod (*Gadus morhua* L.) I — Effects on growth and protein retention. *Aquaculture* 272: 599-611.

[12]. Humason, G.L. (1979). *Animal Tissue Techniques*. W.H. Freeman & Co, San Francisco, 641 pp.

[13]. Poleksić, V., Savić, N., Raškoviċ, B. and Marković, Z. (2006). Effect of different feed composition on intestine and liver histology of trout in cage culture *Biotech in Animal Husbandry* 22: 359-372.

[14]. Andreozzi, G., Antonucci, R., Affatato, C., Gargiulo, G. and Battaglini, P. (1994). *Histo. Embryo.* 23:102-111.

[15]. Kruatrachue, M., Rangsayatorn, N., Pokethitiyook, P., Upatham, E.S. and Singhakaew, S. (2003). Histopathological changes in gastrointestinal tract of fish *Puntius gonionatus* fed on dietary Cadmium. *Bull of Environmental Contamination and Toxicology* 71:

0561-0569.

[16]. Refstie, S., Forde-Skjaervik, O., Rosenlund, G. and Rorvik, K. (2006). Feed intake, growth, and utilisation of macronutrients and amino acids by 1- and 2-year old Atlantic cod (*Gadus morhua*) fed standard or bioprocessed soybean meal. *Aquaculture*, 255: 279-291.

[17]. Gomez-Requeni, P., Mingarro, M., Calduch-Giner, J.A., Medale, F., Martin, S.A.M., Houlihan, D.F., Kaushik, S. and Perez-Sanchez, J. (2004). Protein growth performance, amino acid utilisation and somatotropic axis responsiveness to fish meal replacement by plant protein sources in gilthead sea bream (*Sparus aurata*). *Aquaculture*, 232: 493-510.

[18]. Gatlin, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, Å., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E.J., Stone, D., Wilson, R. and Wurtele, E. (2007). Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquaculture Research* 38: 551-579.

[19]. Chowdhary, S., Srivastava, P.P., Mishra, S., Yadav, A.K., Dayal, R. and Lakra, W.S. (2012). Synergistic effects of dietary glucosamine and plant/animal proteins on the growth performance of asian catfish (*Clarias batrachus*) juveniles *Journal of Animal Feed Research* 2(1): 50-57.

[20]. Uran, P.A., Goncalves, A.A., Taverne-Thielem, J.J., Schrama, J.W., Verreth, J.A.J. and Rombout, J.H.W.M. (2008). Soybean meal induces intestinal inflammation in common carp (*Cyprinus carpio* L.). *Fish & Shellfish Immunol*ogy 25: 751-760.

[21]. Natarajan, A. (1979). Some histopathological and physiological correlates of lead intoxication in the fish, *Barbus stigma*. M. Phil. Thesis, Annamalai University, Annamalai Nagar, India, pp. 143.

[22]. Kumar, S. and Pant, S.C. (1984). Organal damage by Aldicarb to a freshwater teleost *Barbus conchonius* Hamilton. *Bulletin of Environmental Contamination and Toxicology* 33: 50-55.

[23]. Pandey, A.K., Peer Mohamad, M, and George, K.C. (1994). *Journal of Advanced Zoology* 15(1): 18-24.

[24]. Boonyaratpalin, M., Suraneiranat, P. and Tunpibal, T. (1998). Replacement of fish meal with various types of soybean products in diets for the Asian seabass, *Lates calcarifer*. *Aquaculture* 161: 67- 78.

[25]. Krogdahl, A°., Bakke-McKellep, A.M. and Baeverfjord, G. (2003). Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar* L.). *Aquaculture Nutrition* 9: 361–371.

[26]. Heikkinen, J., Vielma, J., Kemila¨inen, O., Tiirola, M., Eskelinen, P., Kiuru, T., Navia-Paldanius, D. and Von Wright, A. (2006). Effects of soybean meal based diet on growth performance, gut histopathology and intestinal microbiota of juvenile rainbow trout (*Oncorhynchus mykiss*).*Aquaculture* 261: 259–268.

[27]. Wang, Y., Kong, L.J., Li, C. and Bureau, D.P. (2006). Effect of replacing fish meal with soybean meal on growth, feed utilization and carcass composition of cuneate drum (*Nibea miichthioides*). *Aquaculture* 261:1307–1313.

[28]. Konar, S.K.(1970). Some effects of sub-lethal levels of heptachlor on rohu, *Labeo rohita* (Hamilton). *Journal of Inland Fisheries Society*, India 2: 51-54.

[29]. Wong, M.H., Luk, K.C. and Choi, K.Y. (1977). The effects of zinc and copper salts on *Cyprinus carpio* and *Ctenopharyngodon idellus*. *Acta Anatomica* 99 (4): 450-454.

[30]. Sastri, K.V. and Gupta, P.K. (1978a). Effect of mercuric chloride on the digestive system of *Channa punctatus*: A histopathological study. *Environmental Research* 16: 270-278.

[31]. Sastri, K.V. and Gupta, P.K. (1978b). Mercuric chloride intoxication in digestive system of *Channa punctatus*. *Journal of Environmental Pathology and Toxicology* 2:443-446.

[32]. Bakthavathsalam, R., Ramalingam, R. and Ramaswamy, A. (1984). Histopathology of liver, kidney and intestine of the fish *Anabas testudineus* exposed to furadan. *Environment and Ecology* 2(4): 243-247.

Corresponding author: Dr. P.P. Srivastava, Fish Biochemistry and Genomics Laboratory, Molecular Biology and Biotechnology Division, National Bureau of Fish Genetic Resources, Canal Ring Road, P.O. Dilkusha, Lucknow-226 002,UP,India (Present Address :Principal Scientist (Biochemistry), Fish Nutrition, Biochemistry and Physiology Division, Central Institute of Fisheries Education, Panch Marg, Off Yari Road, Versova, Mumbai 400 061, MS, India).
E-mail: ppsicar@gmail.com