ISSN: 2347-5129
IJFAS 2013; 1(2):50-55
www.fisheriesjournal.com
Received: 27-11-2013
Accepted: 09-12-2013

Shipra Chowdhary
National Bureau of Fish Genetic Resources, Canal Ring Road, Teli Bagh, Lucknow – 226 002,UP, India.

Prem Prakash Srivastava
National Bureau of Fish Genetic Resources, Canal Ring Road, Teli Bagh, Lucknow – 226 002,UP, India.

Joykrushna Jena
National Bureau of Fish Genetic Resources, Canal Ring Road, Teli Bagh, Lucknow – 226 002,UP, India.

Akhilesh Kumar Yadav
Aquaculture Research Training Unit, National Bureau of Fish Genetic Resources, Chinhat,Faizabad Road, Lucknow-227 105, UP, India.

Rajesh Dayal
National Bureau of Fish Genetic Resources, Canal Ring Road, Teli Bagh, Lucknow – 226 002,UP, India.

Suman Mishra
Department of Applied Animal Science, Babasaheb Bhimrao Ambedkar University, Rae Bareilly Road, Lucknow-226 027, UP, India

S.M. Srivastava
National Bureau of Fish Genetic Resources, Canal Ring Road, Teli Bagh, Lucknow – 226 002,UP, India.

Correspondence
Prem Prakash Srivastava
National Bureau of Fish Genetic Resources, Canal Ring Road, Teli Bagh, Lucknow – 226 002,UP, India.
(Present Address : Fish Nutrition, Biochemistry and Physiology Division, Central Institute of Fisheries Education, Panch Marg, Off Yari Road, Mumbai, MS, India.)

Histological studies of the intestine in threatened Asian catfish (*Clarias batrachus*) fingerlings fed with animal or plant origin protein blended with glucosamine

Shipra Chowdhary, Prem Prakash Srivastava, Joykrushna Jena, Akhilesh Kumar Yadav, Rajesh Dayal, Suman Mishra, Satyendra Mohan Srivastava

ABSTRACT
In the present study a 12-week feeding trials was conducted with animal or plant protein (in combination with Glucosamine source @ 0.0, 0.5%, 5.0% and 10.0%) with threatened Asian Catfish, *Clarias batrachus* (av. wt. 2.2±0.009 to 2.6±0.03 g) to evaluate the effects of proteins of different origin, blended with glucosamine, on intestinal tissues. Intestine of *C. batrachus* fed with natural food (NATFO, F7) showing normal architecture of intestine with circular muscles, longitudinal muscles, serosa and villi. Intestine of fish fed with P:A: G::0:100:0.5 feed (F1) and P:A:G::0:100:5.0 feed (F2) showing normal appearance of circular muscles, longitudinal muscles, serosa and villi. Intestine of fish fed with P:A:G::0:100:10.0 feed (F3) depicting normal appearance of circular muscles, longitudinal muscles, serosa and villi. Fusion of few villi is recorded. Intestine of fish fed with P:A:G::100:0:0.5 feed (F4) showing appearance of spaces between circular muscles and base of villi. Normal circular muscles, longitudinal muscles and serosa are seen. Intestine of fish fed with P:A:G::100:0:5.0 feed (F5) showing elongated lumen in villi. Circular muscles, longitudinal muscles and serosa are normally seen. Intestine of fish fed with P:A:G::100:0:10.0 feed (F6). showing elongated lumen in villi. Circular muscles, longitudinal muscles and serosa are seen normally. However, some detachment of base of villi observed. Results indicate that animal protein diets rich with glucosamine showing normal intestinal tissues architecture than in plant protein fed fishes. The plant protein fed fishes showed an alteration in the intestinal architecture in this threatened fish. However, the results suggests that supplementation of glucosamine has no direct relation with the histological alterations in the fish intestinal tissues.

Keywords: *Clarias batrachus,* dietary protein, histology, intestine.

1. Introduction

In fish production system the nutritionally balanced feed is essential to economically produce healthy and, high quality fish and/ or fish products. Riche and Garling [1] reported that fish reared in intensive tank systems requires all nutrients in a complete pelleted diet. The study for the cellular changes has been elucidated to see the impact, if any, in the tissue on feeding the fish for a longer time on alternative feeds/ ingredients. In this connection, it is necessary to replace fishmeal in diets with less expensive raw materials of plant origin. However, due to the increased fiber content, higher quantity of carbohydrates, anti-nutritional factors, and inappropriate content of amino acids can have negative effects on the digestive system of fish and therefore, on health and production of culturable fish. 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.
The intestine is the most important organs in digestion and absorption of nutrients from food, and therefore, monitoring of these organs is considered necessary [2, 3]. Histological analysis of the digestive system is considered a good indicator of the nutritional status of fish [4-6]. Fishmeal (FM) as raw material is the first choice in aquaculture production due to high quality protein with almost balanced amino acid profile [7, 8]. The aim of the fish feed production is the replacement of FM with less expensive source of protein, 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 [9, 10]. Hardy [11] explained that the industry will soon run out of sufficient quantities of fish oil and meal. Even if all these features are present in plant, it does not mean that it can be used as the complete replacement of FM. It is common that only a part of the proteins in feed is replaced, but even such low level of replacement can have an effect on fish organism, primarily on the digestive system at tissue level. Recent research is pointing out possibilities of almost 100 % replacement of FM in diets for carnivorous fish species like Atlantic salmon and Atlantic cod with proteins of alternative origin without adverse effects on growth [12, 13]. Soybean based products represent a major source of protein in diets for monogastric animals, and many have shown great potential as FM replacements in diets for several fish species [14-20]. Soybean is low in methionine [21, 22]. Air-breeding Asian catfish, *Clarias batrachus* (Family: Clariidae), locally known as Magur, is now an threatened fish and is of great demand and attracts the attention of farmers for its high market value. Since knowledge of the structure of the intestine is essential to the understanding of physiological and also abnormal conditions, this research was undertaken to describe the intestinal histology of *Clarias batrachus*. This experiment was carried to study the synergistic effects of dietary glucosamine in combinations with plant or animal proteins on the histological alteration in the intestine of *Clarias batrachus.*

2. Materials and Methods

Six feeds were prepared using animal & plant proteins in two combinations along with three combinations of glucosamine (GlcN) for feeding to threatened Asian catfish, *C. batrachus*. The animal protein combinations were comprised of three ingredients namely fish meal, silkworm pupae and casein in equal ratio whereas, plant protein comprising of only soybean meal. Out of six-types of feeds developed, three were constituted with only plant based protein and three with equal ratio of animal combination proteins. These six-types of feeds were blended with three combinations of GlcN in 0.5, 5.0 and 10.0 percent share. Thus the ratio of plant protein: animal protein: GlcN in different feeds were F-1, PAG:: 0:100:0.5; F-2, PAG:: 0:100:5.0; F-3, PAG :: 0:100:10.0; F-4, PAG:: 100:0:0.5; F-5, PAG:: 100:0:5.0; F-6, PAG:: 100:0:10.0. The details of feed ingredients used in various feeds and their proximate composition are given in Table- 1.

Table 1: Ingredients composition (w/w) of feeds for *Clarias batrachus* Adult

Feeds / Ingredients	F1	F2	F3	F4	F5	F6	Control
	PAG 0:100:0.5	**PAG 0:100:5.0**	**PAG 0:100:10.0**	**PAG 100:0:0.5**	**PAG 100:0:5.0**	**PAG 100:0:10.0**	**NATFO**
Soybean meal[1]	0.0	0.0	0.0	60.8	60.8	60.8	-
Silkworm Pupae	20.3	20.3	20.3	0.0	0.0	0.0	-
Fish Meal	20.3	20.3	20.3	0.0	0.0	0.0	-
Casein[2]	20.2	20.2	20.2	0.0	0.0	0.0	-
Glucosamine (Chitosamine –HCl)[3]	0.5	5.0	10.0	0.5	5.0	10.0	-
Starch[4]	32.0	27.5	22.5	32.0	27.5	22.5	-
CMC[5]	2.2	2.2	2.2	2.2	2.2	2.2	-
Papain[6]	2.0	2.0	2.0	2.0	2.0	2.0	-
VM + MM[7]	2.5	2.5	2.5	2.5	2.5	2.5	-
Natural -Live food	-	-	-	-	-		100.0
Total	100	100	100	100	100	100	100

P:A:G = Plant Protein : Animal protein : Glucosamine; CMC = Carboxy – methyl – cellulose. [1]HiMedia, Mumbai Lot No: 0000013648; [2] HiMedia, Mumbai Lot No: 0000016171; [3]HiMedia, Mumbai, Lot No: 0000028805; [4]HiMedia, Mumbai, Lot No: 0000028340; [5]HiMedia, Mumbai, Lot No. 0000014218; [6]HiMedia, Mumbai, Lot No. 0000003862; [7]Each kg of Vitamin and mineral mixture named 'Agrimin Forte' contains Vit. A 700000 IU, Vit. D_3 70000 IU, Vit. E 250mg, Nicotinamide 1000mg, Co 150mg, Cu 1200mg, I 325mg, Fe 1500mg, Mg 6000mg, Mn 1500mg, K 100mg, Se 10mg, Na 5.9mg, S 0.72%, Zn 9600mg, Ca 25.5%, P 12.75% Manufacturer Brindavan Phosphates Pvt. Ltd, 48N, doddaballapur Ind. Area, Doddaballapur – 561 203, India Batch No. BFA-61.

The fishmeal was freshly prepared in the laboratory from dried trash fishes comprising mainly of *Mystus vittatus*, and *Puntius sophore*. Live silkworm pupae were procured from Department of Applied Animal Science, Babasaheb Bhimrao Ambedkar University, Raebareli Road, Lucknow, reared up to VI Instar larvae and de-oiled by di-ethyl-ether (Merck). The de-oiled pupae was dried in oven at 60 ºC for an hour, powdered and used for feed preparation. The feeds were prepared by thoroughly mixing the dry ingredients in a mixer and subsequently water was added to make stiff dough. Each feed was cooked in a pressure cooker for 15minutes for proper gelatinization of the ingredients. Finally cooked moist feeds were stored in plastic zipped polybags in a deep freezer (-20 ºC) until used. The newly hatched larvae of *C. batrachus* was obtained from a single batch of hatchery spawned brooders for the experiment and acclimatized for one week to experimental conditions in a wet laboratory. The experiment was taken up in 14 circular plastic pools (7 feed types with 2 replicates) of capacity 300 litre, each containing 50 litre of pre-settled water from a ground aquifer. In each tank 100 fry of *C. batrachus* (av. wt. 2.2$\pm$0.009 to 2.6$\pm$0.03 g) were kept and reared continuously for 84 days. The experimental feeds were hand-fed @ 10% of the total body weight. Each scheduled daily ration per batch of fish was divided into two equal proportions and distributed to the fish at 11:00 hr and 17:00 hr respectively. Initial and subsequent fortnightly weight gains (g) were recorded on electronic balance (make: Sartorius). At the end of the experiment, 6-8 fish from each treatment were sacrificed and analyzed for proximate composition of the muscles.

After twelve weeks of the feeding experiment the animals were sacrificed. The distal intestine from (NATFO) and experimental fishes (Plant or animal protein fed fishes) were excised and fixed in 4% formaldehyde and processed by standard histological techniques i.e. kept in aqueous Bouin's fluid for 24-h and washed for 8-hr in running tap water. The organs were routinely processed (dehydrated in ethanol series, embedded in paraffin, serially sectioned sectioned at 6 μ).

Sections of the intestine were stained with Haematoxylin and Eosin (HE) Humason [23]. Histological slides were observed under microscope (Labomed, Model: Digi 2).

3. Results

Fishes fed with P:A:G::0:100:0.5 feed (F1) showing normal appearance of circular muscles, longitudinal muscles, serosa and villi (Photo-2). Intestine of *Clarias batrachus* fed with P:A:G::0:100:5.0 feed (F2) showing normal appearance of circular muscles, longitudinal muscles, serosa and villi (Photo-3). Intestine of *Clarias batrachus* fed with P:A:G::0:100:10.0 feed (F3) depicting normal appearance of circular muscles, longitudinal muscles, serosa and villi. Fusion of few villi seen (Photo -4).

The *Clarias batrachus* fed with P:A:G::100 :0:0.5 feed (F4) showing appearance of spaces between circular muscles and base of villi. Normal circular muscles, longitudinal muscles and serosa are seen (Photo-5). Intestine of *Clarias batrachus* fed with P:A:G::100:0:5.0 feed (F5) showing elongated lumen in villi. Circular muscles, longitudinal muscles and serosa are normally seen (Photo-6). Fishes fed with P:A:G::100:0:10.0 feed (F6) showing elongated lumen in villi. Circular muscles, longitudinal muscles and serosa are seen normally. Showing some detachment of base of villi observed (Photo-7). The *Clarias batrachus* fed F7 with natural feed i.e. *Artemia nauplii* and minced chicken alimentary canal meat showing, normal architecture of intestine with circular muscles, longitudinal muscles, serosa and villi (Photo-1). Results of F1-F6 are compared with the control.

In future, experiments in the area of fish nutrition, histological state of intestine should always be taken into account. This should provide additional information about state of this organ if any of mentioned methods are used. These methods are valuable in field experiments, as well as in the laboratory.

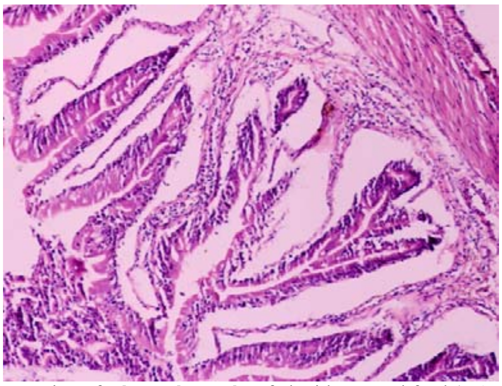

Plate 1: Intestine of *Clarias batrachus* fed with Natural feed (NATFO, F7) showing normal architecture of intestine with circular muscles, longitudinal muscles, serosa and villi. H/E **X 125**.

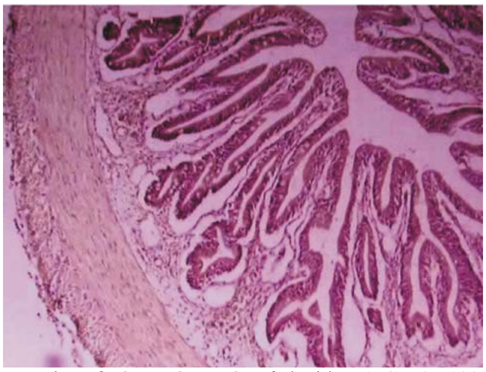

Photo - 2 Intestine of *Clarias batrachus* fed with P:A:G :: 0 : 100 : 0.5 feed (F1) showing normal appearance of circular muscles, longitudinal muscles, serosa and villi. H/E **X 125**.

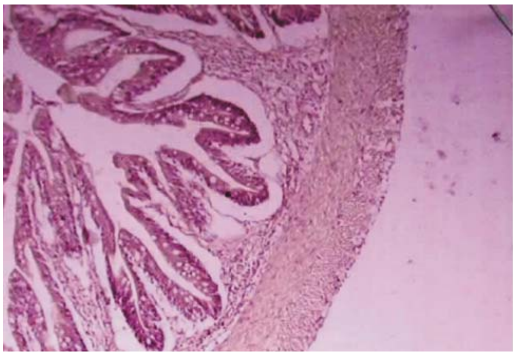

Photo 3: Intestine of *Clarias batrachus* fed with P:A:G :: 0 : 100 : 5.0 feed (F2) showing normal appearance of circular muscles, longitudinal muscles, serosa and villi. H/E **X 125**.

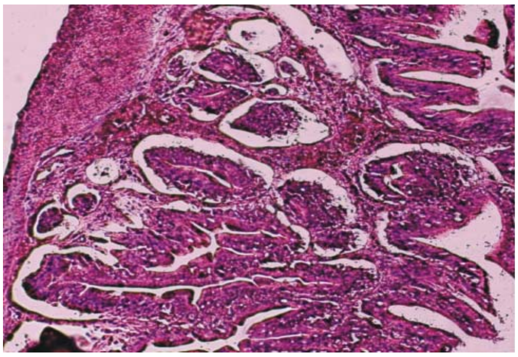

Photo 4: Intestine of *Clarias batrachus* fed with P:A: G :: 0 : 100 : 10.0 feed(F3) depicting normal appearance of circular muscles, longitudinal muscles, serosa and villi. Fusion of few villi seen. H/E **X 125**.

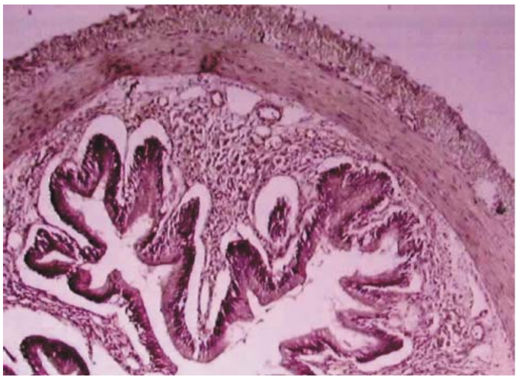

Photo 5: Intestine of *Clarias batrachus* fed with P:A:G :: 100 : 0 : 0.5 feed (F4) showing appearance of spaces between circular muscles and base of villi. Normal circular muscles, longitudinal muscles and serosa are seen. H/E **X 125**.

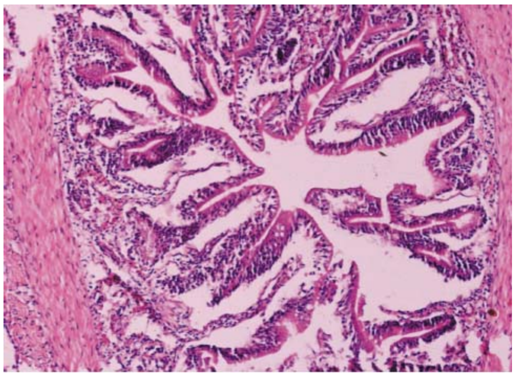

Photo 6: Intestine of Clarias batrachus fed with P:A:G :: 100 : 0 : 5.0 feed (F5) showing elongated lumen in villi. Circular muscles, longitudinal muscles and serosa are normally seen. H/E X 125. H/E **X 125**

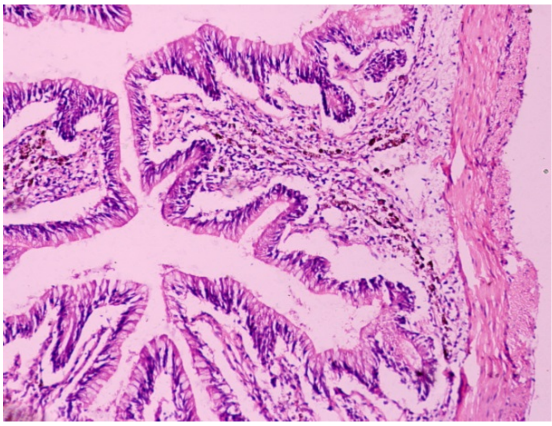

Photo 7: Intestine of *Clarias batrachus* fed with P:A:G :: 100 : 0 : 10.0 feed(F6). Showing elongated lumen in villi. Circular muscles, longitudinal muscles and serosa are seen normally. Showing some detachment of base of villi observed. H/E **X 125**.

4. Discussion
Effects of dietary ingredient on histology of the intestine were investigated. Histological structure of this part of the digestive system is: mucosa, which consists of the lamina epitelialis (simple columnar and glandular epithelium) and lamina propria (connective tissue); submucosae, consisting of two layers (stratum compactum and stratum granulosum); muscular layer and serosa [24]. This histological structure is not altered even in experiments in which fish were fed food containing heavy metals [25, 26]. 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 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 [27, 28], where weight was depressed up to 30% in the group that had 100% replacement of FM with plant proteins. Hansen *et al*. [13] 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 FM by proteins of plant origin [7]. The finding of Chowdhary *et al*. [29] indicates that animal protein rich feeds with glucosamine were much acceptable than natural feeds for Asian catfish, *Clarias batrachus*. Uran *et al*. [30] reported that carp show signs of enteritis when fed high levels of soy in the diet.

In the present study, marked histopathological changes in the intestine of *Clarias batrachus* have been observed, proliferation of villi, and necrosis of serosa, mucosa and sub-mucosa as well as space in villi necrosis and fusion of villi. The intestinal wall of *Clarias batrachus* comprised of four distinct layers, viz. mucosa, submucosa, muscularis and serosa. The mucosal layer being thrown into finger like villi, which is made up of simple, long columnar cells and numerous goblet cells (mucous cells) with centrally placed nuclei. Sub-mucosa is thin and projected into mucosal folds constituting the lamina propria. This layer is composed of loose connective tissue with numerous collagen fibres and blood cells. Muscularis consists of inner, thick, circular, and outer, thin, longitudinal muscular layers. Serosa is formed of peritoneal layer and blood capillaries. Fishes fed with P:A:G::0:100:0.5 feed (F1) showing normal appearance of circular muscles, longitudinal muscles, serosa and villi. Intestine of *Clarias batrachus* fed with P:A:G::0:100:5.0 feed (F2) showing normal appearance of circular muscles, longitudinal muscles, serosa and villi. Intestine of *Clarias batrachus* fed with P:A:G::0:100:10.0 feed (F3) depicting normal appearance of circular muscles, longitudinal muscles, serosa and villi. The *Clarias batrachus* fed with P:A:G::100:0:0.5 feed (F4) showing appearance of spaces between circular muscles and base of villi. Normal circular muscles, longitudinal muscles and serosa are seen. Intestine of *Clarias batrachus* fed with P:A:G::100:0:5.0 feed (F5) showing elongated lumen in villi. Circular muscles, longitudinal muscles and serosa are normally seen. Fishes fed with P:A:G::100:0:10.0 feed (F6) showing fusion of few villi and the results are in corroboration with the findings of other authors [31-33]. Observations made by earlier workers relating to histopathological changes in intestine in response to various protein feeds are being enumerated here. The reduced growth in fish may be due to anti-nutritional factors [9, 34, 35]. The histological changes in intestine can also reduce growth performance on feeding plant proteins [36-39]. The proliferation, necrosis of serora and mucosa and rupture of villi have been reported by Konar [40] and in *Labeo rohita*; Wong *et al*. [41] in *Cyprinus carpio* and *Ctenopharyngodon idellus;* Sastri and Gupta [42, 43] in *Channa punctatus*; Kumar and Pant [32] in *Barbus conchonius;* Bakthavathsalam *et al*. [44] in *Channa punctatus* against exposure to heptachlor, zinc and copper salt mercuric chloride, dimecron, aldicarb and furadan, respectively. The replacement of 30% and more has been critical for digestion [45]. Increasing the percentage of soybean meal in feed led to the increased intensity of enteritis [37, 46, 47] evaluated four practical diets with different FM content (0, 15%, and 30%). In this the fishmeal was fully and/or partly replaced with ingredients of non-animal origin and supplemented with methionine and lysine. Effects on growth and histological structure of liver and intestine were investigated by the above authors [37,46,47]. They recorded as normal morphology in carp distal intestine. However, some slides showed leucocytes infiltration in the epithelium, most often accompanied by increased mucous production, regardless of dietary treatment. Feed containing solely plant protein sources had the highest fold length and the lowest growth rate. In the present study the effects of soybean based proteins represent a major source of protein in diets for monogastric animal and may have shown great potential as fishmeal replacement in the diets for several fish species has been well studied. The replacement of animal protein by plant animal and graded levels of glucosamine is acceptable from growth point of view and in relation to minor changes in intestine morphology and/or histological architecture. The inclusion of glucosamine through diet does not impacted in histological alterations in the intestine tissue architecture.

5. Acknowledgements
Authors are very grateful to the Director, NBFGR, Lucknow for providing facilities to conduct this research work.

6. References
1. Riche M, Garling D. Feeding Tilapia in intensive re-circulatory systems. North Central Regional Aquaculture Centre and United State Department of Agriculture USDA, 2003; 1-4.
2. Takashima F, Hibiya T. An atlas of fish histology: normal and pathological features. Kodansha, distributed by Fischer, G., Tokyo, 1982; 147.
3. Roberts RJ. Fish pathology. Baillière Tindall, London, 1989.

4. Hall KC, Bellwood DR. Histological effects of cyanide, stress and starvation on the intestinal mucosa of *Pomacentrus coelestis*, a marine aquarium fish species. Journal of Fish Biology 1995; 47:438-454.
5. Green BS, McCornick MI. Influence of larval feeding history on the body condition of *Amphiprion melanopus.* Journal of Fish Biology 1999; 55:1273-1289.
6. Caballero MJ, Izquierdo MS, Kjørsvik E, Montero D, Socorro J, Fernández AJ *et al.* Morphological aspects of intestinal cells from gilthead seabream (*Sparus aurata*) fed diets containing different lipid sources. Aquaculture 2003; 225:325-340.
7. Gatlin DM, Barrows FT, Brown P, Dabrowski K, Gaylord TG, Hardy RW *et al.* Expanding the utilization of sustainable plant products in aquafeeds a review. Aqua Res 2007; 38:551-579.
8. Watanabe T. Strategies for further development of aquatic feeds. Fisheries Science 2002; 68:242-252.
9. Francis G, Makkar HPS, Becker K. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 2001; 199:197-227.
10. Marković Z, Poleksić V, Dulić Z, Stanković M. 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 2009; 498-499.
11. Hardy RW. Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. Aqua Res 2010; 41:770-776.
12. Espe M, Lemme A, Petri, A, El-Mowafi A. Assessment of lysine requirement for maximal protein accretion in Atlantic salmon using plant protein diets. Aquaculture 2007; 263:168-178.
13. Hansen AC, Rosenlund G, Karlsen O, Koppe W, Hemre GI. Total replacement of fish meal with plant proteins in diets for Atlantic cod (*Gadus morhua* L.) I — Effects on growth and protein retention. Aquaculture 2007; 272:599-611.
14. Refstie S, Helland SJ, Storebakken T. Adaptation to soybean meal in diet for rainbow trout *Oncorhynchus mykiss.* Aquaculture 1997; 153:263-272.
15. Refstie S, Korsoen OJ, Storebakken T, Baeverfjord G, Lein I, Roem AJ. Differing nutritional responses to dietary soybean meal in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). Aquaculture 2000; 190:49-63.
16. Storebakken T, Refstie S, Ruyter B. Soy-products as fat and protein sources in fish feeds for intensive aquaculture. In: J. K. Drachley (Ed.), Federation of Anim Sci Soc, Savoy 2000; 127–170.
17. Fagbenro OA, Davies SJ. Use of soybean flour (dehulled, solvent-extracted soybean) as a fish meal substitute in practical diets for African catfish *Clarias gariepinus* (Burchell 1822): growth feed utilization and digestibility. Jour of Appl Ichthyol 2001; 17:64-69.
18. Romarheim OH, Skrede A, Penn M, Mydland LT, Krogdahl A, Storebakken T. Lipid digestibility bile drainage and development of morphological intestinal changes in rainbow trout (*Oncorhynchus mykiss*) fed diets containing defatted soybean meal. Aquaculture 2008; 274:329-338.
19. Schuchardt D, Vergara JM, Fernandez-Palacios H, Kalinowski CT, Hernandez-Cruz CM, Izquierdo MS, Robaina L. Effects of different dietary protein and lipid levels on growth feed utilization and body composition of the red porgy (*Pagrus pagrus*) fingerlings. Aqua Nutr 2008; 14:1-9.
20. Ali A, Al-Ogaily SM, Al-Asgah NA, Goddard JS, Ahmed SI. Effect of feeding different protein to energy (P/E) ratios on the growth performance and body composition of *Oreochromis niloticus* fingerlings. Jour of Appl Ichthyol 2008; 24:31-37.
21. Pongmaneerat J, Watanabe T. Nutrition evaluation of soybean meal for rainbow trout and carp. Nippon Suisan Gakkaishi 1993; 59:157-163.
22. Schwarz FJ, Kirchgessner M, Deuringer U. Studies on the methionine requirement of carp (*Cyprinus carpio*). Aquaculture 1998; 161:121-129.
23. Humason GL. Animal Tissue Techniques. W.H. Freeman & Co, San Francisco, 1979; 641.
24. Poleksić V, Savić N, Rašković B, Marković Z. Effect of different feed composition on intestine and liver histology of trout in cage culture. Biotech in Anim Hus 2006; 22:359-372.
25. Andreozzi G, Antonucci R, Affatato C, Gargiulo G, Battaglini P. Influence du cadmium sur l`intestin de *Carassius auratus*. Anatomia Histologia Embryologia 1994; 23:102-111.
26. Kruatrachue M, Rangsayatorn N, Pokethitiyook P, Upatham ES, Singhakaew S. Histopathological changes in gastrointestinal tract of fish *Puntius gonionotus* fed on dietary Cadmium. Bull of Environ Contam and Toxicol 2003; 71:0561-0569.
27. Refstie S, Forde-Skjaervik O, Rosenlund G, Rorvik K. 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 2006; 255:279-291.
28. Gomez-Requeni P, Mingarro M, Calduch-Giner JA, Medale F, Martin SAM, Houlihan DF, Kaushik S, Perez-Sanchez J. 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 2004; 232:493-510.
29. Chowdhary S, Srivastava PP, Mishra S, Yadav AK, Dayal R, Lakra WS. Synergistic effects of dietary glucosamine and plant/animal proteins on the growth performance of Asian catfish (*Clarias batrachus*) juveniles. J Anim Feed Res 2012; 2(1):50-57.
30. Uran PA, Goncalves AA, Taverne-Thielem JJ, Schrama JW, Verreth JAJ, Rombout JHWM. Soybean meal induces intestinal inflammation in common carp (*Cyprinus carpio* L.). Fish & Shellfish Immunol 2008; 25:751-760.
31. Natarajan A. Some histopathological and physiological correlates of lead intoxication in the fish, *Barbus stigma*. M. Phil. Thesis, Annamalai University, Annamalai Nagar, India, 1979; 143.
32. Kumar S, Pant SC. Organal damage by Aldicarb to a freshwater teleost *Barbus conchonius* Hamilton. Bull Environ Contam Toxicol 1984; 33:50-55.
33. Pandey AK, Peer Mohamed M, George KC. Centl Marine Fisheries Res Inst (ICAR), PB No. 1603, Cochin-682 014). Histopathological alterations in liver and intestine of *Liza parsia* (Hamilton-Buchanan) in response to mercury toxicity. J Adv Zoo 1994; 15(1):18-24.
34. Leenhouwers JI, Adjei-Boateng SD, Verreth JAJ. Digesta viscosity, nutrient digestibility and organ weights in

African catfish (*Clarias gariepinus*) fed diets supplemented with different levels of a soluble non-starch polysaccharide. Aquaculture Nutrition 2006; 12:111–116.
35. Ye J, Liu X, Wang Z. Effect of partial fish meal replacement by soybean meal on the growth performance and biochemical indices of juvenile Japanese flounder, *Paralichthys olivaceus*. Aquacult Int 2011; 19:143-153.
36. Boonyaratpalin M, Suraneiranat P, Tunpibal T. Replacement of fish meal with various types of soybean products in diets for the Asian seabass, *Lates calcarifer*. Aquaculture 1998; 161:67-78.
37. Krogdahl A, Bakke-McKellep AM, Baeverfjord G. 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 2003; 9:361–371.
38. Heikkinen J, Vielma J, Kemila¨inen O, Tiirola M, Eskelinen P, Kiuru T *et al.* Effects of soybean meal based diet on growth performance gut histopathology and intestinal microbiota of juvenile rainbow trout (*Oncorhynchus mykiss*). Aquaculture 2006; 261:259-268.
39. Wang Y, Kong LJ, Li C, Bureau DP. Effect of replacing fish meal with soybean meal on growth, feed utilization and carcass composition of cuneate drum (*Nibea miichthioides*). Aquaculture 2006; 261:1307-1313.
40. Konar SK. Some effects of sub-lethal levels of heptachlor on rohu, *Labeo rohita* (Hamilton). Journal of Inland Fisheries Society India 1970; 2:51-54.
41. Wong MH, Luk KC Choi KY. The effects of zinc and copper salts on *Cyprinus carpio* and *Ctenopharyngodon idellus.* Acta Anatomica 1977; 99(4):450-454.
42. Sastri KV, Gupta PK. Effect of mercuric chloride on the digestive system of *Channa punctatus*: A histopathological study. Environmental Research 1978; 16:270-278.
43. Sastri KV, Gupta PK. Mercuric chloride intoxication in digestive system of *Channa punctatus*. Journal of Environmental Pathology and Toxicology 1978; 2:443-446.
44. Bakthavathsalam R, Ramalingam R, Ramaswamy A. Histopathology of liver, kidney and intestine of the fish *Anabas testudineus* exposed to furadan. Environment and Ecology 1984; 2(4):243-247.
45. Baeverfjord G, Krogdahl A. Development and regression of soybean meal induced enteritis in Atlantic salmon, *Salmo salar* L., distal intestine: A comparison with the intestines of fasted fish. Journal of Fish Diseases 1996; 19:375-387.
46. Urán PA, Schrama JW, Jaafari S. Variation in commercial sources of soybean meal influences the severity of enteritis in Atlantic salmon (*Salmo salar,* L.). Aquaculture Nutrition 2009; 15:492-499.
47. Marković Z, Poleksić V, Lakić N. Evaluation of Growth and Histology of Liver and intestine in Juvenile Carp (*Cyprinus carpio*, L.) Fed Extruded Diets with or without Fish Meal. Turkish Journal of Fisheries and Aquatic Sciences 2012; 12:1-2.
48.