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

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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::100:0:5.0 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::100:0:10.0 feed (F3) depicting normal appearance of circular muscles, longitudinal muscles, serosa and villi. Fusion of few villi is recorded. In 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 normally seen. 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]. Fishmeal (FM) as raw material is the first choice in aquaculture production due to high quality protein with almost balanced amino acid profile [5, 6]. 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 characteristic attributes 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 synergic 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                          | F1              | F2              | F3              | F4              | F5              | F6              |
| Ingredients                    | PAG 0:100:0.5   | PAG 0:100:5.0   | PAG 0:100:10:0  | PAG 0:100:0.5   | PAG 0:100:5.0   | PAG 0:100:10.0  |
| Soyworn Meal                   | 0.0             | 0.0             | 0.0             | 60.8            | 60.8            | 60.8            |
| Fish Meal                      | 20.3            | 20.3            | 20.3            | 0.0             | 0.0             | 0.0             |
| Casein                         | 20.3            | 20.3            | 20.3            | 0.0             | 0.0             | 0.0             |
| Glucosamine (Chitosamine –HCl) | 0.5             | 5.0             | 10.0            | 0.5             | 3.0             | 0.0             |
| Starch                         | 32.0            | 27.5            | 22.5            | 32.0            | 27.5            | 22.5            |
| CMG                           | 2.2             | 2.2             | 2.2             | 2.2             | 2.2             | 2.2             |
| Papain                         | 2.0             | 2.0             | 2.0             | 2.0             | 2.0             | 2.0             |
| VM + MM                       | 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             |

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 °C 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 15 minutes for proper gelatination of the ingredients. Finally cooked moist feeds were stored in plastic zipped polybags in a deep freezer (-20 °C) 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±0.009 to 2.6±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.
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 epithelialis (simple columnar and glandular epithelium) and lamina propria (connective tissue); submucosa, 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 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. [32] 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 submucosa 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::0:100:10.0 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; Bakhanaal 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, NBFGAR, Lucknow for providing facilities to conduct this research work.

6. References


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