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ORIGINAL ARTICLE

Study Histological of Intestinal in Fish fed with Hydrolyze of Cow Skin

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ABSTRACT
This study aimed to investigate possible changes the small bowel tissue in the Flower Horn Fish was fed with hydrolyze of cow skin. 216 fish with average weight and length, 0.95 ± 0.2 (g) and 3.7 ± 0.2 cm respectively, using diets six of isocaloric and formulated by the software 2/8 of win Feed with different percentages including (zero, 20, 40, 60, 80 and 100), hydrolyze protein of cow skin in the form treatments six, each with three replicates were examined. During the experimental period were 100 days, and fish bioassays were once every 20 days. Fish treatment 40% and treatment 100% demonstrate the best performance and the lowest increase of growth (increased length and weight) Compared to the control group (P<0/05), respectively. The results of the small intestine samples of 54 fish (3 fish per replicate) showed that the overall situation of intestine tissue in of the fish were fed with of 20%, 40% and 60% levels hydrolyze of cow skin, was healthy similar to control treatment. But in the intestinal tissue of fishes have been fed with 80% and 100% of hydrolyze of cow skin, symptoms such as fusing some intestinal tissue and damage of Serosa layer was seen.
Key words: nutrition, intestinal histology, hydrolyze of cow skin, Flower horn fish.

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INTRODUCTION

hydrolyze of cow skin is part of the cow skin inner layer that containing 68% protein and essential amino acids such as methionine, tryptophan, lysine, histidine, valine, leucine, arginine, proline, serine, and cysteine. The annual world production of this substance was approximately 230000 tons, in 2013. In recent years, In Iran manufacturer's aquatic and poultry feed in different cities, with method of acid hydrolyze of 85% have begun to produce and use it, and now monthly 60-40 tons of this material produced in the country. Due to the Fish meal expensive and unreliable of the aquatic resources for the production of it, hydrolyze protein of cow skin can be a good alternative to fish meal in rations and thus reduces production costs of ration. Different types of food that can be used by fish, directly on the function and morphology of some body tissues especially affect the intestinal tissue [8]. Intestinal tissues when exposed to contact with different foods, show the different responses that with investigate of this changes can be discovered to size nutrient function in the body [9]. Flower Horn fish belongs to the family Cichlidae is from the world's most popular aquarium species. The male generator of fish from Cichlid and specie *Amphilophus citrinellus* is with commercial name of Midas and female generator of it's from Cichlid and specie *Cichlasoma trimaculatum* with commercial name of Trymako [3].

MATERIAL AND METHODS

In this study from 18 tank, fish 216 of Flower Horn with average weight and initial length 0.95 ± 0.2 (g) and 3.7 ± 0.2 cm respectively, that were produced from one parent and were have the health file, in the form of treatment six, each with 3 repeat, entirely randomly were abandoned in tanks (12 of fish per tank). Drinking water was used to source of water supply. The temperature measured daily and the amount of oxygen with meter oxygen model laboratory device (JENWAY 970) before bioassays at 8

o'clock of morning and pH with a meter pH device model (DEVER UB-10) were measured weekly. The average temperature of the water workshop 27 ± 1°c, dissolved oxygen is 6.5 ± 0.5 mg/l, pH is 7.1±0.5 and the hardness of the water were 168±1.5 mg/l, respectively. In order to make proper food ration with suitable of rate protein and calories, hydrolyzed powder of cow skin by Pravar Newin Company and with method of acidy hydrolyze 85% was produced, and along with the fish meal of Clupeonella cultiventris caspia produced by Behparvar Company, to determine the approximate composition of the materials, was transported to the lab. And the amount of protein with using Kjeldahl method, crude fat according to Soxhlet method, and moisture, ash and carbohydrates, as well as to the procedure provided by the AOAC [1] were measured (table 1).

Table 1. Approximate percentage of fish flour and hydrolyze of cow skin used in research.

raw materials	protein	fat	ash	moisture	fiber	Carbohydrate
Fish meal	57	7.2	14.7	10	0.1	0.1
hydrolyze of cow skin	68.25	3.25	17	3.52	0.05	0.1

rations Six of isocaloric with specific levels of hydrolyze of cow skin include: one-treatment (T1) contains 20%, the two-treatment (T2) contains 40%, the three-treatment (T3) contains 60%, the four- treatment (T4) containing 80%, the five-treatment (T5) contains 100% of hydrolyze of cow skin and control treatment (T6) containing 0% of hydrolyze of cow skin with the use of raw materials as demonstrated in the table (2) and on the basis of the formula preparation by the software of win feed$\frac{1}{2}$ was produced.

Table (2). Fish food ration components built for Flower Horn fish based on the percentage, by the software *of* win feed $\frac{2}{8}$ have been formulated.

Food treatment / raw Materials (%)	T_1 (20%)	T_2 (40%)	T_3 (60%)	T_4 (80%)	T_5 (100%)	T_6 (control treatment)
Fish flour of Clupeonella cultiventris caspia	29.42	23.17	16.06	7.61	0	34.83
Wheat flour	13.64	12.62	21.2	11.47	11.58	10.58
Hydrolyze of cow skin	7	16.8	24.29	28.64	31.32	0
Wheat gluten	25.7	24.33	19.95	21.58	30.9	33.7
fish oil of clupeonella cultiventris caspia	4	4.1	4.5	4.75	6.6	6.9
Additives *	16	17.92	16	16	18.4	16
Di-ca phosphate	3.4	3.3	2.8	2.55	1	-

* Additives include:3% binder , 1% lysine, 1% methionine, 2% astaxanthin – 0.5% antifungal, 2.5% Mineral premixed, vitamin Mineral premixed of 3%, 1% antioxidants.

At the end of feeding period, randomly the fish nine were selected from different treatments (three per each tank) to prepare samples of intestine. Selected fishes transferred to Histology lab of Imam Khomeini located in Karaj city and were anesthetized with clove extract 50 ppm [5]. Then the intestinal tissues were isolated and for 48 hours in formalin 10% as a fixative, were fixed. Preparation of microscopic sections and coloring was performed with H&E approach according to standard method of [10] and prepared sections were examined using an optical microscope manufactured by REICHERT-JUNG Company [5].

RESULTS

At the end of the experiment period, feuded fishes with rations containing 40% (T2) with a weight increase average of 2.88 ± 0.52 g had the highest rate of increase and difference was significant compared to with other treatment ($P<0.05$). The lowest rate of weight increase was related to T5 with an average of 1.66 ± 0.62 g. Flower Horn fish survival rate at five treatments fed with hydrolyze of cow skin in comparison with the control group showed no significant difference at the end of the growing period ($P<0.05$). In investigate samples of intestine different treatments with control treatment, Fusing of the intestinal villi in the treatment T4 (figure 2-e) and the damage Serosa layer in treatment T5 (Figure 1- f) were observed. The intestinal tissue of fishes treatment T1 (Figure1-a), while the control treatment (figure 1-b), treatment T2 (figure 1-c), and treatment T3 (Figure1-d) were not specific sign and significant, and intestinal tissues of fishes in this study were healthy, and there was no dramatic morphology changes and serious tissue damage in the building of intestinal.

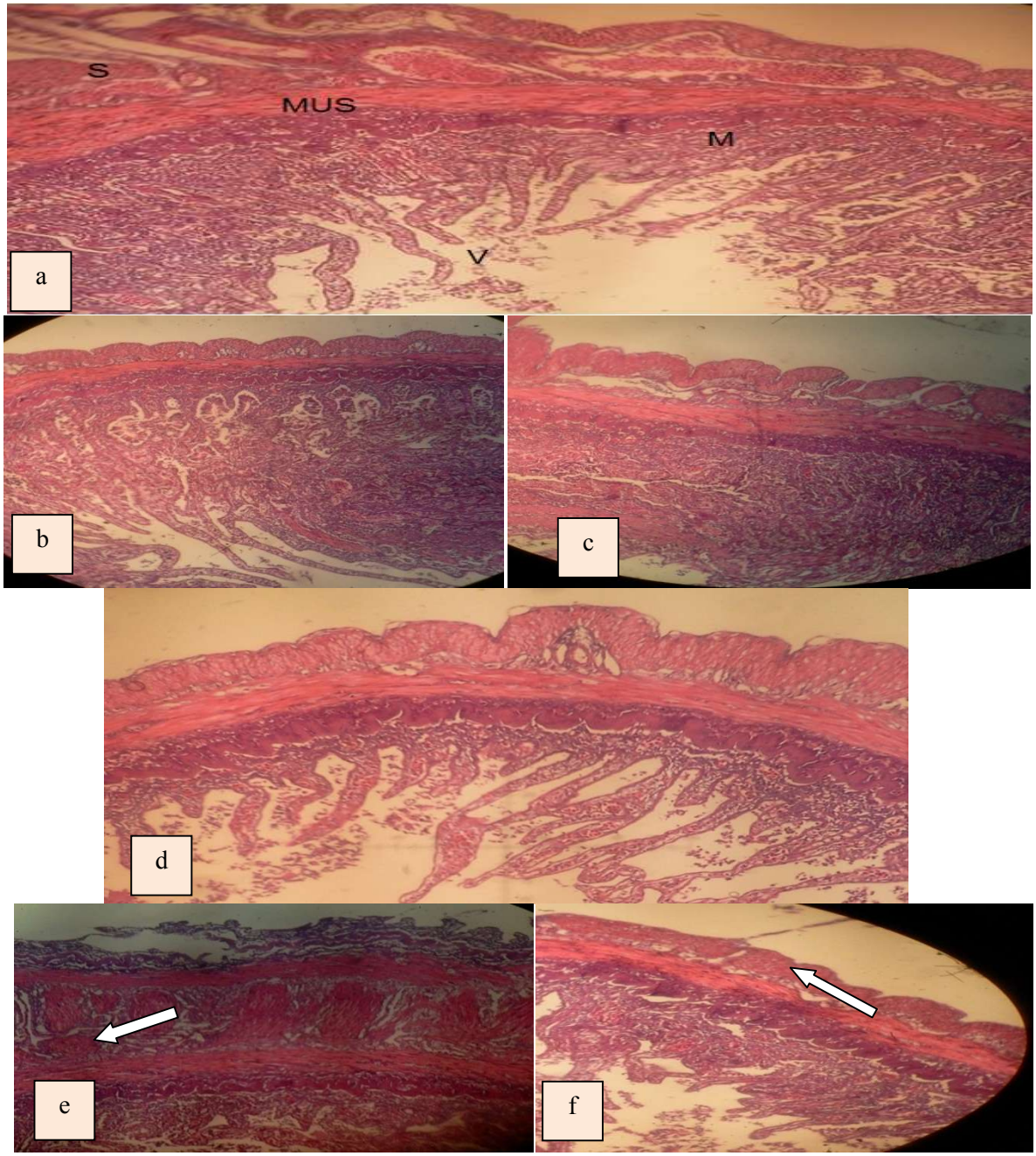

Figure 1. The intestinal tissue in the flower Horn fishes fed with different levels of hydrolyze of cow skin. a) No perceptible effect, M = the mucous layer, S= Serosa layer, SM = SM = the mucous below layer, MUS = muscle layer, layer, b, c and d) No perceptible effect, e) fusing villi (white arrows), f) serosalayer injury. Zoom = ×10.

DISCUSSION AND CONCLUSIONS

Evaluation of intestinal tissue of fishes, in this study indicates that generally, the intestinal tissue, in feeding fishes with the lower than levels of 60% hydrolyze of cow skin, compared to intestinal tissue feed the fishes with higher levels, was healthier. In spite of the vast volume of studies in the field of vegetable and animal food different effects with a variety of Add-ons and compounds on aquatic creatures, so far histology test based on the effect of hydrolyze of cow skin on the fishes intestinal tissue has not been reported. In the study by Evans et al [6] took place, Histological changes of the small intestine of tubules fishes (Punctatusictalurus) with diets containing raw soybean flour, and treatmented soybean flour with different temperatures were fed, examined. The results showed that between treatments, there are not significant differences sensible of intestine tissue. Rumsey et al [11] with study on the salmon that with

diets containing 30-25% soybean flour were fed showed that Between the initial and final part of the small intestine from view of cell swelling, change in the number of vacuoles in epithelial tissue and changes in the intestinal villi, There are significant differences sensible. Based on the views of Rumsey, amount of cell swelling and number of Vacuoles in epithelial tissue of the intestine Compared to proximal reduced, but thickness of the villi in the proximal significantly associated with increased. The Atlantic fish (*Hippoglossus hippoglossus*) that were fed diets containing 36% soybean flour, Morphological and histological changes considerably compared to control treatments were found in the intestinal tissue [7].
In a study performed by Buttle et al [2] concluded that some compounds such as Lectin, glycine and B-conglycinin in the incidence of tissue lesions in the small intestine involved and are causes swelling of the cells. Also, some compounds are solvable in alcohol such as saponins, or some oligo-saccharides in addition to the changes in the tissues of the spleen, pancreas and digestive glands, can cause swelling of cell in intestine tissue [12]. Edith et al [4] examined the effects of methyl parathion toxic at doses lower than the fatal level of it combined with food for ornamental fishes *Corydoras paleatus* on intestinal tissue. The results demonstrated that 2 hours after food digestion Goblet cell numbers were significantly decreased compared with the control diet ($P<0.05$) and After 8 hours of food digestion lipoid vacuoles located at the top enterocytes vanished and disappeared. Small intestinal epithelium tissue was severe necrotic, and was accompanied with lymphocytes swelling and entrocytes necrosis.

REFERENCES

1. AOAC. (1990). Official methods of analysis, 14th edition Association of Official Analytical Chemists, Arlington, VA., 1102pp.
2. Buttle, L.G., Burrells, A.C., Good, J.E., Williams, P.D., Southgate, P.J. &Burrells, C. (2011). The binding of soybean agglutinin (SBA) to the intestinal epithelium of Atlantic salmon, Salmon salar and rainbow trout, Oncorhynchusmykiss, fed high levels of soybean meal. Vet. Immunology.Immunopathology.80,237–244.
3. Dadgar, SH., Akbari, H., Sarpanah, E., (2012). Atlas of fresh-water aquarium fish.Mojesabze Publications, Tehran, 22pp.
4. Edith, f., Flavia, s., Silvia, r., Ana, c., Sander, f., (2003).. Histology of the fish Corydoras paleatus contamined with sublethal Levels of organo phosphorus in water and food. Ecotoxicology and environmental safety.54: 119-130pp.
5. Ellif. I. C., (2000). Gill and kidney histopathology in the fresh water fish Cyprinuscarpio after aqute exposure to deltamethrin. Environmental toxicology and Pharmacology.22: 200-204.
6. Evans, DH. Piemarini, PM., and Choe, KP.,(2005). The multifunctional fish gill dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous, waste. Phsiol Rev.85:97-177.
7. Grisdal-Helland, B., Helland, S.J., Baeverfjord, G. & Berge, G.M. (2012). Full-fat soybean meal in diets for Atlantic halibut: growth, metabolism and intestinal histology.Aquacult.Nutr. 8,265–270.
8. Heikkinen R., Vihriälä E., Vainionpää A., Korpelainen R., Jämsä T. (2007). Acceleration slope of exercise-induced impacts is a determinant of changes in bone density. J. Biomech. 40, 2967–297410.1016/j.jbiomech.2007.02.003 [PubMed] [Cross Ref].
9. Munira, N., Mohammad, A., and Ashraf, U., (2009). Histopathological changes in the gill, liver and intestine of Heteropneustesfossilis (Blooch) treated with extracts of different parts of the plants Madhuca Indica (G.F.Gmel).4:113-119.
10. Posty, A., Moradi, A., (2001). Comparative histology and histopathology techniques. Tehran University Press, 531pp.
11. Rumsey, G.L., Endres, J.G., Bowser, P.R., Earnest-Koons, K.A., Anderson, D.P. & Siwicki, A.K. (1995). Soy protein in diets of rainbow trout: effects on growth, protein absorption, gastrointestinal histology and nonspecific serologic and immune response. In: Nutrition and Utilization Technology in Aquaculture (Lim, C. &Sessa, D.J. eds) pp. 166–188. AOCS Press, Champaign, IL, USA.
12. Van den Ingh, T.S.G.A.M., Olli, J.J. &Krogdahl, A. (1996).Alcohol soluble components in soybeans cause morphological changes in the distal intestine of Atlantic salmon, Salmosalar L. J. Fish Dis., 19, 47–53.