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Effects of L-Lysine on growth and histological changes in liver and intestine of GIFT Strain *Oreochromis niloticus*

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Abstract. Four experimental diets were used for four treatments *viz.*T_1 (SD+1.95% lysine of diet), T_2 (SD+2.05% lysine of diet), T_3 (SD+2.15% lysine of diet) and T_4 (Control, SD+0.00% lysine of diet) with each having three replications to understand the effects of lysine on growth, histological changes in liver and intestine of GIFT strain of tilapia (*Oreochromis niloticus*). Forty-eight days old tilapia fry were stocked in experimental cemented tanks and fed twice a day with experimental diets containing different doses of L-lysine for 40 days from May to June 2016 and then the growth and health status of experimental fish were checked at every 10 days interval. The mean body weight treatment T_2fish (13.04±0.3 g) was significantly higher ($p<0.0001$) followed by T_3(10.58±0.14g), T_1(9.86±0.07 g) and T_4, (7.79±0.05 g), respectively. In addition, the body length was also increased 8.63±0.81 cm with T_2 treatment followed by 8.59±0.08 cm, 8.42±0.12 cm and 8.1±0.06 cm with T_3, $T_1$and T_4, respectively. SGR of fish were 2.41±0.004, 2.52±0.04, 245±0.07 and 2.07±0.04% in T_1, T_2, T_3 and T_4, respectively. Histology of liver and intestine was carried out with T_1, T_2, T_3 and T_4 treatment where the microvillus fold and height in the intestine were comparatively higher in T_2, but there were no vacuoles and infiltrations in T_2 compared to T_1, T_3 and T_4. The present findings revealed that 2.05% supplementary lysine for tilapia might be a good promoter for their growth and culture.

Keywords: Lysine, GIFT, Histological changes

Introduction

Protein is the most expensive component in fish feed and also the most important nutrient for better growth performance. The selection of proper quantity and quality of dietary protein is a necessary tool for successful tilapia culture practices. However, information on the gross protein requirement is of limited value without data on the essential amino acid (EAA) requirements because protein quality depends on its amino acid composition and digestibility. Lysine is an EAA required by all fish species investigated to date. Lysine has one major function of protein deposition in the animal body. It is the second most limiting amino acid (Robinson and Li 2007). Lysine helps absorption of calcium, maintain healthy blood vessels, produces antibodies, enzymes, collagen and repairs tissues. A typical commercial production diet formulated for tilapia or catfish contains approximately 32-40% protein (Miles and Chapman 2008). Fish do not have a specific protein requirement but rather a definite requirement for essential amino acids that comprise proteins. Lysine is the most important amino acid, accounting for 7.2% of protein. Lysine is the first limiting essential amino acid in many protein sources used for feed and lysine-rich ingredients are often expensive. In global livestock production lysine is also considered as one of the fastest growing factors

(FAO 2012). It plays an important role in cell division, healing of wounds, removing ammonia from the body, immune function, and release of hormones. Dietary lysine supplementation may result in the weight gain, nitrogen retention and reduction in body fat content of fish. Lysine participates in a single metabolic pathway targeted for muscle growth by hyperplasia or recruitment and enlargement of muscles fibers by hypertrophy and elongation.

National Research Council (1993) reported that *O. niloticus* required 14 g lysine/kg diet while Corazon and Richard (1988) observed that *O. niloticus* required 14.3 g lysine/kg diet. *O. mossambicus* need 16 g lysine/kg diet; *Ictalurus punctatus* 12 g lysine/kg diet; *Clarias gariepinus* 23 g lysine/kg diet and *Cyprinus carpio* 22 g lysine/kg diet. Further to this, they reported that the efficiency of lysine utilization for whole body protein deposition was affected both by lysine and digestible energy levels in the diet.

Research on dietary nutrient requirements and supplementation of different nutrients has been studied for tilapia. However, very few works have been conducted on the effects of lysine on growth and histological changes of liver and intestine of GIFT strain of tilapia. Simultaneously, many fish feed industries have been developed and expanding day by day. Therefore, the knowledge on the effects of lysine supplementation on growth and histological changes in liver and intestine is very important. Considering the above facts, the present study was conducted to investigate the effect of lysine on the growth performance and observe histological changes in the liver and intestine of tilapia.

Materials and Methods

Study area

Rectangular shaped twelve cemented tanks facilitated to supply water from motor were used for the study in the laboratory of Fish Biology and Genetics Department, Sylhet Agricultural University for 40 days from 12th May to 22nd June 2016.

Sample collection

Forty eight days old fries of tilapia were collected from Pirojpur Fish Nursery in Sylhet district and acclimated to the laboratory conditions for 2 days prior to feeding trial for their adaptation to experimental diet.

Experimental design

The experiment was conducted in four treatments each with three replications having almost the same sized 50 experimental fish. The design of experiment is shown in Table I. The treatments have been designated as T_1(1.95% lysine), T_2(2.05% lysine), T_3(2.15% lysine)and T_4 (0.00 % lysine), respectively.

Diet preparation and feeding schedule

Supplemental diet was prepared according to Hasan (2015) with minor modifications (Table I). The feed was applied carefully twice a day during the 40 days experimental period. Half of the ration was supplied at 9.00 am and the remaining half was supplied at about 5.00 pm. Fish were fed at a rate of 20% of their body weight. Then the rate was gradually reduced to 12%.

Table I. Composition of the formulated feed containing a different concentration of lysine

Component	T_1	T_2	T_3	T_4
Rice bran	21%	21%	21%	21%
Wheat bran	15%	15%	15%	15%
Wheat flour	10%	10%	10%	10%
Fish meal	40%	40%	40%	40%
Maize meal	13%	13%	13%	13%
Vit-B complex	0.5%	0.5%	0.5%	0.5%
Vit-E	0.5%	0.5%	0.5%	0.5%
Total	100	100	100	100
Total L- Lysine	1.95%	2.05%	2.15%	0.00%

Physico-chemical parameter

Water quality parameters such as water temperature, pH, dissolved oxygen (DO), alkalinity, the concentration of ammonia, nitrite, nitrate and water transparency were measured at every 10 days interval. Temperature and DO were measured by a digital DO meter (YSI model 58) and pH with a pH electrode (Jenway, model 3020). Before taking a measurement, pH meter was properly adjusted with buffer solution (pH-7). Water quality parameters (temperature 25-29°C, dissolved oxygen ≥ 6 ppm, and pH7-7.5) were recorded between 08.00-09.00 am. Other parameters were monitored by HACH kit.

Experiment procedure

At the end of the experiment, the fish were harvested by draining out the cemented tanks. Individual weight and length data were recorded and different growth parameters were calculated according to the following formula:

Length gain (cm) = Mean final length (cm)– Mean initial length (cm)

Weight gain (g)= Mean final weight (g) – Mean initial weight (g)

$$\% \text{ of weight gain} = \frac{\text{Weight gain (g)}}{\text{Initial weight (g)}} \times 100$$

$$\% \text{ of length gain} = \frac{\text{Length gain (cm)}}{\text{Initial length (cm)}} \times 100$$

Specific growth rate (SGR, %/day) = [(Ln final weight − Ln initial wt.) /duration] × 100

$$\text{Hepato Somatic Index(HSI)}(\%) = \frac{\text{Weight of liver (g)}}{\text{Weight of body (g)}} \times 100$$

Histological observation of liver and intestine

Histological study of the liver and intestine of tilapia was conducted according to the standard protocol. Briefly, the samples were kept in Bouin's fixatives for 12-24 hours. Then the fixed specimens were processed to wax embedded blocks using a vacuum infiltration processor and standard protocols (Jabed 2013). Sections were cut at 3-5 μm on a motorised rotary microtome, mounted on glass slides, dried and stained with haematoxylin and eosin (H&E). Stained sections were observed under compound light microscope and photographed by using an attached digital camera (Olympus Xcam-Alpha, Germany).

Statistical analysis

Finally, the collected data were analyzed statistically by using IBM SPSS statistics, version 19.

Results and Discussion

Final weight and length

After the 40 days of the feeding trial, final weights of fish were 9.86 ± 0.07g, 13.04 ± 0.03 g, 10.58 ± 0.14 g and 7.79 ± 0.05 g in T_1, T_2, T_3 and T_4, respectively (Table II). Final weights were influenced by dietary lysine supplementation and a significantly higher final weight was found in T_2(13.04 ± 0.30g). However, the final length of *O. niloticus* varied among the treatments and it was not significantly influenced by dietary lysine supplementation. The mean final lengths were 8.42 ± 0.12 cm, 8.63 ± 0.81 cm, 8.59 ± 0.08 cm and 8.1 ± 0.06 cm in T_1, T_2, T_3 and T_4 respectively (Table II). Ovie and Ere (2013) observed increased weight gain in tilapia fingerlings with the supplementation of dietary lysine. The current study strongly supports the above findings. In another study for Japanese flounder Han *et al.* (2013) indicated that fish fed with 2.05 g lysine/100 g diet could induce a higher weight gain than the other diet groups. Lower supplementation level often causes reduced growth performance (Han *et al*. 2013, Alam *et al*. 2002) which is also reflected in the present study. Alam *et al.* (2002) reported lesser growth rate fed with lower lysine supplementation (1.65g/100g diet) in Japanese flounder compared to the higher lysine groups (higher than 2.05g/100g diet). The increased growth rate of fish due to lysine supplementation might be caused due to its effect on growth factor gene expression. Banos *et al.*(1999) reported that injection of lysine to brown trout *Salmo trutta* significantly stimulated the expression of insulin-like growth factor-I (IGF-I) in plasma where the main role of IGF-I is the regulation of development and growth by mediating growth hormone action.

In the present study, lysine supplementation increased the growth rate up to 2.05% lysine supplementation (T_2). The reduced growth rate in T_3 might be due to the excess level of lysine in the diet. Halver (2002) reported that overdose of lysine can cause the

weight loss of tilapia. The increased growth performances also might be due to enhanced feed and protein utilization of GIFT strain. Although protein utilization was not measured in the present study, however, enhanced feed utilization was reflected by reduced FCR. Lysine supplementation helps to improve blood chemical parameters which support better health condition as well as the better growth of fish (Han *et al.* 2013).

Specific Growth Rate (SGR)

The recorded SGR values in the present study were 2.41±0.38, 2.52±0.26, 2.45±0.17 and 2.07±0.29 in T_1, T_2, $T_3$and T4, respectively. The SGR was significantly higher in T_2 where the diet contained 2.05% lysine supplementation. Fish of T_3 showed intermediate value and it was not significantly different from T_1. The significantly lower value was obtained in T_4 (Table II). The results of the present study agree with the previous findings (SGR values between 2.03 to 2.30) of Green (1992) and Hossain *et al.* (2004) for the Nile and GIFT tilapia, respectively. Ahmed and Khan (2004) also found more or less similar SGR values (1.55 - 2.46) for *Cirrhinus mrigala*. The little variance of SGR in the current study might be due to the variation of the experimental condition, as we conducted the experiment in cemented tanks.

Viserosomatic Index (VSI)

The viserosomatic index (VSI) of experimental tilapia significantly varied among the treatments. The VSI of tilapia were 5.36±0.31, 8.81±0.28, 6.17±0.66 and 5.13±0.21 in T_1, T_2, T_3 and T_4, respectively (Table II). The significantly higher VSI was found in T_2(8.81±0.28g) and it did not differ with T_3. The significantly lower value was obtained in the control group (T_4) which did not differ with 1.95% lysine supplementation (T_1).

Hepatosomatic Index (HSI)

The hepatosomatic index (HSI) of tilapia ranged from 1.12 to1.66. HIS values were not significantly influenced by dietary lysine supplementation. However numerically higher HSI was found in T_2 (1.66±0.15) and the lowest in T_4 (1.12±0.31) (Table II). The liver size is related to the nutrition status of the fish (Shoemaker *et al.* 2003). In the current study numerically increased HSI with lysine supplementation indicates proper storage of macro and micronutrients and healthy condition of the liver as well as clinically healthy fish.

Table II. Growth parameters and viserosomatic index of *O. niloticus* after the 40-day experimental period fed with different doses of lysine supplemented feed

Treatment	Final length (cm)	Final weight (g)	Specific growth rate (%)	Viserosomatic Index (VSI)	Hepatosomatic index (HSI)
T_1	8.42±0.12	9.86±0.07	2.41± 0.38	5.36±0.31	1.46±0.15
T_2	8.63±0.81	13.04±0.03	2.52 ±0.26	8.81±0.28	1.66±0.15
T_3	8.59±0.08	10.58±0.14	2.45 ±0.17	6.17±0.66	1.53±0.25
T_4 (Control)	8.1±0.06	7.79±0.05	2.07 ±0.29	5.13±0.21	1.12±0.31

Histological observation of fish liver and intestine

Histological analysis of the digestive system is considered to be a good indicator of the nutritional status of fish (Hall and Bellwood 1995, Green and Mc Cornick 1999). The intestine and liver are the most important organs for digestion and absorption of nutrients from food, and therefore monitoring of these organs is considered necessary (Takashima and Hibiya 1982). A few noticeable changes in liver and intestine histology were observed in the present study compared to the previous related research by Hasan (2015).

In T_1 (containing 1.95% supplemented lysine), the observed liver and intestinal tissue developments were very poorly. Vacuoles were less and infiltrations were high in the liver tissues (Fig. 1); villi were not developed in the intestine (Fig. 2). The highest villi length of intestine was measured 26.15µm after 40 days feeding trial. In T_2 (containing 2.05% supplemented lysine) the observed liver and intestinal tissue of the experimental fish were developed clearly. Both infiltrations and vacuoles were less in the liver (Fig. 3); development of villi in the intestine was clearly visualized (Fig. 4). The highest villi length of intestine was measured 41.18µm after 40 days feeding trial. In T_3 (containing 2.15% supplemented lysine) the liver and intestinal tissue development were less than that in T_2. Vacuoles were high and infiltrations were more. Liver cells were not observed clearly and the cyst was observed (Fig. 5). In T_3 the highest villi length of intestine was measured at 29.06µm (Fig. 6). In T_4 (containing 0.00% supplemented lysine) the observed liver and intestinal tissue development were very poor; liver cells were compact with each other and vacuoles were formed among the cells (Fig. 7). In this treatment, the highest villi length of intestine was measured at17.68µm (Fig. 8).

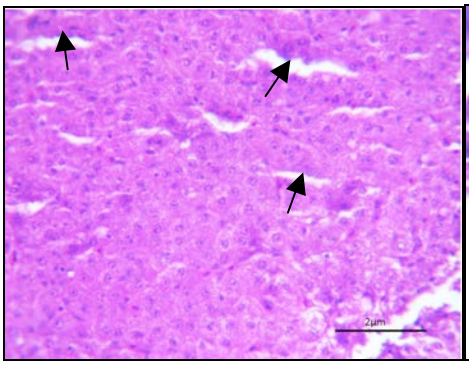

Fig. 1. Liver in T_1 after 40 days.

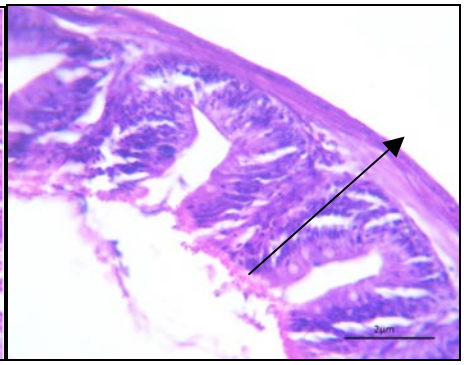

Fig. 2. Intestine in T_1 after 40 days.

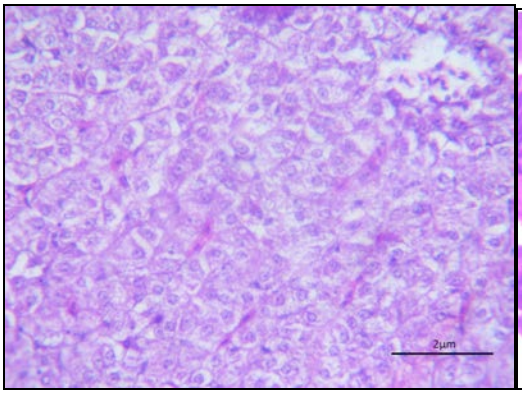

Fig. 3. Liver in T_2 after 40 days.

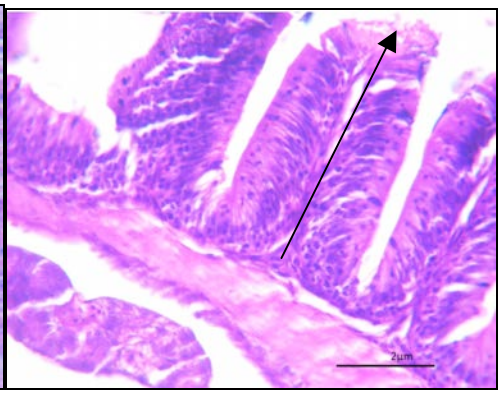

Fig. 4. Intestine in T_2 after 40 days.

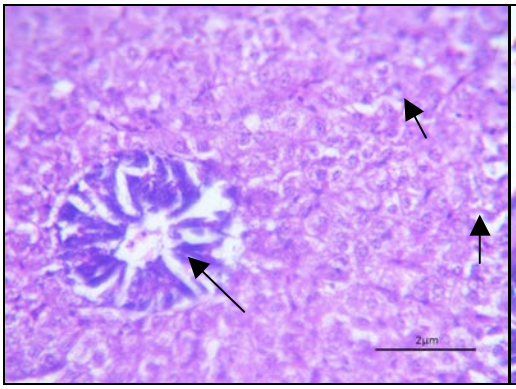

Fig. 5. Liver in T_3 after 40 days.

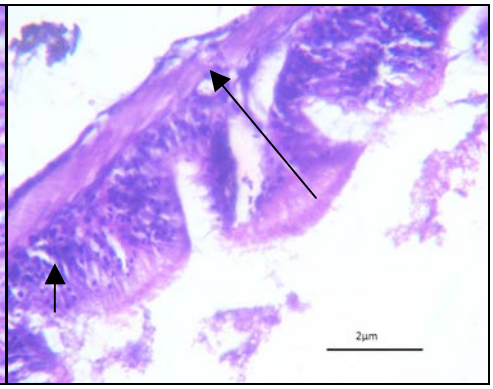

Fig. 6. Intestine in T_3 after 40 days.

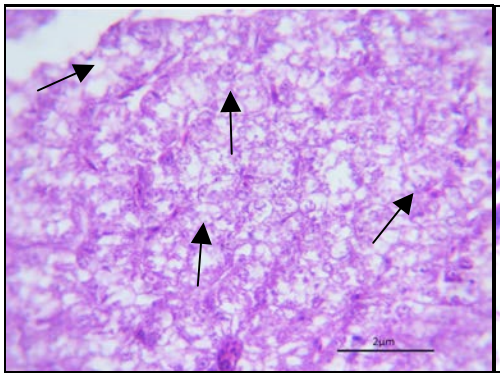

Fig. 7. Liver in T_4 after 40 days.

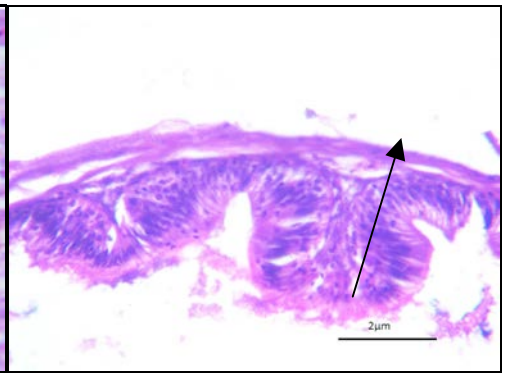

Fig. 8. Intestine in T_4 after 40 days.

The histological sections of the intestine of the tilapia fish exhibited simple columnar epithelium, lamina propria, submucosa, tunica muscularisand serosa as well-defined layers in the order from internal to the external surface of the intestine. The epithelial cells appeared as long and thin columnar cells and the mucosa showing surface microvilli. Histological changes in the intestine may vary depending on the species and feed used in the experiment.

From the histological observations of liver and intestine of *O. niloticus* different deformities were observed due to different doses of lysine. Histological changes in the liver are easily recognized if the food used is not adequate (Tacon 1992). The most common changes observed in the liver are: hepatocytes vacuolization, fatty degeneration of the liver, changes in metabolic activity, changes in liver parenchyma and necrosis (Takashima and Hibiya 1982). In T_2, liver and intestinal condition were good and well developed, all the villi were clearly visualized in the intestine and the liver showed many center filtrations in this treatment. The highest villi length was measured, 41.18µm in T_2. In the other treatments (T_1, T_3 and T_4), many vacuoles and infiltration were observed.

Lysine has influenced the body weight and length and also internally liver weight and intestine weight. Final weights were significantly influenced by dietary lysine supplementation and significantly higher final weight was found in T_2. T_3 showed intermediate value followed by T_1. The significantly lower value was found in T_4 (control).

Conclusions

The results from the present study confirm the effects of lysine on the performances of GIFT strain *O. niloticus*. Supplementation of lysine also enhanced the feed efficiency of tilapias as well as promote the development of the intestine and liver. Lysine plays a significant role in increasing body length and weight. This also helped in liver and intestine weight and development of the experimental fish. In respect of body length and weight, liver weight and intestine weight, SGR, HIS, and VSI T_2(2.05% lysine of diet) showed the best performance whileT_3(2.15% lysine) showed intermediate performance (less than T_2 but higher than T_1 and T_4). This might be due to the overdose of lysine. Thus, from the above findings, it can be concluded that incorporation of 2.05%lysine in tilapia feedmay improve the growth, liver and intestinal health status of GIFT strain of tilapia which will ultimately help to increase the tilapia (GIFT strain) production.

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