

NUTRITIONAL EFFICACY OF ACID FISH SILAGE IN *LABEO ROHITA* AT GROW OUT STAGE

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ABSTRACT

Fish acid silage was prepared by minced fish body viscera and formic acid and its efficacy was studied in *Labeo rohita* at grow out stage. Three iso-nitrogenous diets (treatments), containing 32% crude protein (CP) was prepared using this silage. In the treatment-1 (T₁) diet, only fish acid silage was used, treatment-2 (T₂) diet were prepared by using 75% fish silage and 25% soybean meal and rice bran while 50 % acid silage and 50% soya bean meal and rice bran were used in treatment-3 (T₃) diet. In the control diets (T₀) fish silage was replaced with fishmeal. The experiment was conducted in 12 fiber glass tanks having dimensions 36' x 36' x 24' (Length× width× depth) and stocking density of 15 fish/ tank. There were three replicates for each of the treatment and control diet and the fish in all the tanks were fed @ 4% of their body weight twice daily. Non-significant differences in weight gain, FCR and SGR were observed between T₀ and T₂ diets. The proximate analysis of the carcass showed significantly higher protein content for T₂ and T₀ diets. Similarly, higher fats and ash content were observed for T₂ diets. Significantly higher WBCs and platelets were noted in the blood of fish fed with T₂ diets. Histological studies showed normal liver functioning similarly renal tubules and glomerulus in kidneys were also not affected with any diet. The intestine showed hypercalcia for all the four diets. It can be concluded from the present study that fish acid silage is nutritious product that has no adverse effects on body organs and can be used to prepare cost-effective fish feeds.

Key words: Proximate analysis, Fish body viscera, Growth, *Labeo rohita*, Blood analysis

INTRODUCTION

Pakistan is an agricultural country and the inhabitants depend largely on livestock sector for their protein requirements (Govt. of Pakistan, 2002-2003). The population of the country is increasing at an unprecedented rate and the sole livestock industry is unable to meet protein demand of the masses. The need of the day is to find out some alternates that are economically viable and can fulfill the increasing protein demands (Young, 2001). Aquaculture can play a pivotal role in this direction as protein from fish is cheaper and nutritional value of fish is versatile as compared to other food sources (World Nutrition Forum, 2006). Fish products are of immense importance because fish oil contains eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Saoud *et al.*, 2008; Rafflenbeul, 2001). Besides, fish meat is rich in vitamins like A, D, E and K. These vitamins are fat soluble and help in human metabolism (Kinsella, 1987). To increase fish production, there is dire need to shift the traditional extensive rearing system practices to intensive culture systems. In Pakistan, the most culture able fish species

are Indian major carps i.e. *Labeo rohita*, *Cirrhinus mrigala* and *Catla catla*. These species are hardy, have good market value due to their better taste and good flesh quality and these are preferred by local communities (ICLARM, 2001).

Fish industry in Pakistan is facing many challenges and the biggest among them is the cost of feed. Fishmeal is considered as key ingredient in fish feed formulations but fishmeal is costly and is not readily available (Kureshy *et al.*, 2000). Efforts were made to replace fishmeal with soybean meal to reduce cost of fish feeds however plant origin proteins contain anti-nutritional components that affect growth rate of fish (NRC, 2011). The issue however can be resolved by proper fish waste management. Fish waste in the form of skin, fins, scales, body viscera, head and bones (Wang *et al.*, 2011) makes nearly 45% of live body weight of fish and it is estimated that about 70.3 million tons of waste is generated annually (FAO 2010). This waste deteriorates water quality and is toxic to the aquatic and terrestrial organisms (Duan *et al.*, 2009). Fish waste contains large amount of nutrients in the form of protein, fats, carbohydrates (Rustad, 2003) and a large amount of enzymes. Preservation of these nutrients can result in

valuable products. The preservation techniques include biological and acidic silage methods which result in liquid or paste type product and may contain crude protein up to 30-55% and crude fat up to 8-10% (Ayadi *et al.*, 2012).

Fish silage may be a better choice (Lim *et al.*, 2008) which is a liquid product and can be prepared using fish body wastes like trash fishes or fish body viscera. Different methods like acid silage, biological silage and enzymatic silage can be used to prepare fish silage (Pérez, 1995) and the prepared silage can be used for animal food products (Aryanta *et al.*, 1991), as bio fertilizers (Synnes and Opstadt, 1995) and for human consumption as well (Mackie *et al.*, 1971). Among Indian major carps, *Labeo rohita* is preferred by the local people due to its delicious taste and low price and makes nearly 60% of total fish market (Rafique, 2000). However, the information regarding effect of fish acid silage on growth, hematology, histology and carcass composition of *Labeo rohita* grow out is scarce. The present project was therefore designed to prepare acidic silage from fish body viscera and to determine its efficacy for *Labeo rohita* at grow-out stage, which is the stage at which fingerlings are shifted in ponds or tanks and fed on special diets to accelerate their growth rate for market size.

MATERIALS AND METHODS

The initial weight of fish was 18.56 ± 4.76 g and length was 11.75 ± 2.22 cm for treatment-1 (T_1) diets while initial weight for treatment-2 (T_2) diets was 16.6 ± 3.71 g and length was 16.4 ± 1.22 cm. Similarly the initial weights for treatment-3 (T_3) and control (T_0) diets were 19.42 ± 3.53 g and 16.15 ± 4.21 g and lengths were 12.1 ± 2.11 cm and 13.1 ± 2.15 cm, respectively.

Preparation of fish silage: To prepare acid silage, fish body viscera was collected from Fish Farm, Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Ravi Campus during December, 2013 through February, 2014. The collected body viscera were washed with tape water to remove mud and other debris. Minced fish body wastes were transferred in three plastic tubs and mixed with 3% (v/w), 85% concentrated formic acid following (Geron *et al.*, 2007). Anti-oxidant ethoxyquine (1%) was then added to decrease oxidation of lipids. The mixture was kept at room temperature for 30 days and monitored on daily basis. The excessive fat layers appearing on the surface of the mixture were removed and the mixture was stirred twice daily. The pH was also monitored and maintained between the ranges from 3.5 to 4. After 30 days, brown colored liquid silage with strong fishy smell was obtained.

Nutritional and anti-nutritional analysis: Nutritional quality of the prepared silage was analyzed through

proximate analysis following AOAC (2000). Protein contents were determined by Kjeldhal method, total lipids were obtained by ether extracts, total ash were determined by furnace burning and moisture contents were determined by oven drying. The aflatoxins levels were analyzed following Joshua (1993) and microbial load was checked following El-Shafai *et al.* (2004). Experimental diets were then prepared to check the nutritional efficacy of prepared silage for fish growth.

Preparation of experimental diets: Four iso-nitrogenous diets were prepared with varying concentrations of fish silage. These treatment diets were termed as T_1 , T_2 , T_3 and T_0 . Soybean meal and rice bran were used as co ingredients to formulate fish feeds having 32% protein level. T_1 diets contained 100% fish acid silage, T_2 diets were prepared using 75% fish silage and 25% rice bran and soybean meal. T_3 diets contained 50% fish silage and 50 % rice bran and soybean meal while fishmeal was used as a major feed ingredient in place of fish silage to prepare control (T_0) diets.

Warm water was added to make homogenous paste for all the feed formulations used during present study. This paste was then used to make pellets of 3 mm size through pellet mill. The pellets were oven dried at 40 °C for 48 hours and were kept in sealed polythene bags for further use.

Feeding and growth: This 3-months trial was conducted from May, 15 through August 15, 2014 in twelve rectangular fiber glass tanks having dimensions 36" x 36" x 24" (length x width x depth). These tanks were filled with 200 liters of water and 15 fish were stocked in each aquarium having separate aeration facilities. The initial weight of *Labeo rohita* for T_1 was 18.56 ± 4.76 g and length was 11.75 ± 2.22 cm, For T_2 the initial weight was 16.6 ± 3.71 g and length was 16.4 ± 1.22 cm. For T_3 the weight was 19.42 ± 3.53 g and length was 12.1 ± 2.11 cm while for T_0 the weight was 16.15 ± 4.21 g while the length was 13.1 ± 2.15 cm.

Pelleted feed was offered to fish at 4% of body weight twice daily during dawn and dusk hours. The water quality parameters viz. temperature, dissolved oxygen, pH, salinity and total dissolved solids were recorded on daily basis while gain in fish weight measured using electric weighing balance and fish length using ordinary scale fortnightly.

At the end of trial, percentage weight gain, specific growth rate growth, feed conversion ratio and survival (%) were calculated using following formulae;

$$\text{Percentage weight gain} = \frac{\text{Final weight} - \text{initial weight}}{\text{Initial weight}} \times 100$$

$$\text{Specific growth rate (SGR)} = \frac{\text{Ln (Final weight)} - \text{Ln (Initial weight)}}{\text{Experimental periods in days}} \times 100$$

$$\text{Food conversion ratio (FCR)} = \frac{\text{Feed given (dry weight)}}{\text{Body weight gain (wet weight)}}$$

$$\text{Survival (\%)} = \frac{\text{Total number of animal harvested}}{\text{Total number stocked}} \times 100$$

Hematology, histology and carcass composition: For hematological analysis, blood was taken from caudal fin and by puncturing fish heart in 5cc syringe and preserved in two tubes, the first containing disodium salt of ethylene diamine tetra-acetic acid (EDTA) and the other without EDTA. Standard hematological procedures described by Brown (1993) and Blaxhall and Daisley (1973) were employed for the assessment of WBC, RBC, platelets, hemoglobin and growth hormones.

At the end of trial, three fish from each aquarium were sacrificed for histo-pathological analysis following Roberts (1978). The carcass composition of experimental fish was determined following AOAC (2000).

Statistical analysis: The obtained data was analyzed through statistical software SAS 9.1 and Analysis of Variance (ANOVA) was applied to find out differences between means of different treatments.

RESULTS AND DISCUSSION

At the end of thirty days, a material dark brown in color and pasty type liquid was found in tubs with strong fishy smell. It was concluded that fish silage is easy to produce with simple technology. Fish silage is generally a product of high biological value presenting practically the same composition as the original raw material (Tacon, 1993) easy to produce and involves simple artisanal technology. pH between 3 and 3.75 are satisfactory for the preservation of fish residues and similar results were described by Toledo *et al.* (2009) who described that pH<4.5 guarantee the good quality of the silages in this type of byproduct. The pH of the silage prepared during present study was 3.52 and the acid silage having pH between 3 and 4 is relatively stable if stored at ambient storage temperature between 16°C to 30°C (Wassef, 1990).

The proximate analysis indicated that moisture contents of the silage, prepared values were recorded as 4.89±0.57, 6.69±0.43, 5.08±0.83 and 6.05±0.66 protein contents were recorded as 31.88±0.52, 32.23±0.95, 33.10±0.29 and 32.81±1.21. According to Santana-Delgado *et al.* (2008) autolysis effect affects protein content of prepared fish silage. The proteins are converted in to amino acids and peptides that may be unstable during storage periods. Fat contents were recorded as 7.53±1.32, 9.40±0.63, 6.69±0.52 and 4.75±0.84 while ash contents were recorded as 6.58±0.60,

8.41±0.76, 8.79±0.58 and 5.70±0.50 in T₁, T₂, T₃ and T₀, respectively. Statistical analysis revealed that moisture, fat and ash contents varied significantly (p<0.05) in all the four treatments while non-significant differences were recorded in protein content of these treatments. Our findings are contradictory to the findings of González and Marín (2005) and Gerón *et al.* (2007) who analyzed silages prepared from residues of sardines, tilapias and complete Spanish mackerel. Their results for proximate analysis of silages prepared from different residues varied in protein, moisture and fat contents. This difference in proximate analysis values can be justified as described by Vidotti *et al.* (2002b) and Santana-Delgado *et al.* (2008) who stated that these divergences in values of proximate analysis are due to the chemical composition of the different raw materials and nutritional values of raw materials used for acid silage preparation. The species of the fish utilized, sex of the fish, its reproductive status and even the cut at the time of processing affect the chemical composition of silages.

Among anti-nutritional factors, microbial load and aflatoxins were checked out during present study. The results indicated that microbial load and total coliform count was within limit as recommended by APHA (1995). The pure silage was found to be aflatoxins free while minute quantity of Aflatoxins B₁, B₂, G₁ and G₂ was found in all the four treatments diets (Anfossi *et al.*, 2011). During present study, significantly higher weight gain was recorded in T₂ as compared to T₁ and T₃ tanks while non-significant differences in weight gain were observed between T₂ and T₀. The weight gain in T₁, T₂, T₃ and T₀ tanks was recorded as 27.76±2.12g, 40.63±1.43g, 34.85±1.61g and 42.34±1.21g, respectively. Similarly, higher specific growth rate and better feed conversion ratios were observed for fish fed with T₂ than rest of the treatment diets while non-significant variations in SGR and FCR were recorded for T₂ and T₀ diets (Table 1 and 2).

The differences within results of the current study between different treatments may be due to the lower energy content as compared with fishmeal (4102 vs. 4700 Kcal/kg, respectively) Takeuchi *et al.* (2002) reported that energy content in aqua diets are determinant factors that govern feeding and growth efficiency of fish especially during the early life stages. Therefore, fish diets for early stages of fish must be designed carefully to satisfy the optimum levels of fish at this stage.

The availability of amino acids in the co-dried silage based diets may be low because of the destruction of amino acids as a result of the Maillard reaction and fish acid silage contains free amino acids which may be susceptible to this reaction due to which the biological activity of lysine and other amino acids is reduced (Berg and Storebakken, 1996).

Table 1: Nutrient and anti-nutrient evaluation of formulated diets based on different ratios of acid silage

Parameters	T1	T2	T3	T0
Moisture %	4.89±0.57b	6.69±0.43a	5.08±0.83b	6.05±0.66ab
Protein %	31.88±0.52a	32.23±0.95a	33.10±0.29a	32.81±1.21a
Fats %	7.53±1.32b	9.40±0.63a	6.69±0.52b	4.75±0.84c
Ash %	6.58±0.60b	8.41±0.76a	8.79±0.58a	5.70±0.50b
Microbial load cfu/g10 ⁴	1.48± 0.22a	1.34± 0.18a	1.38±0.06a	1.24± 0.06a
Coliform cfu/g10 ⁴	0.93×± 0.04a	0.83± 0.01b	0.72± 0.04c	0.83×± 0.04b
Aflatoxin B1 ppb	0±0.00d	2.53±0.08c	4.66±0.18a	3.31±0.08b
Aflatoxin B2 ppb	0±0.00d	8.85±0.43b	12.28±0.84a	7.34±0.96c
Aflatoxin G1 ppb	0±0.00c	7.23±1.00ab	7.93±0.52a	6.21±0.30b
Aflatoxin G2 ppb	0±0.00c	5.51±0.64b	6.90±0.58a	5.51±0.63b

Means with similar letters in a row are statistically non-significant.

Table 2: Weight gained Length gain, FCR, SGR, and survival rate of *Labeo rohita* fingerlings

Treatments	Total weight gain	Total length gain	FCR	SGR	Survival %
T ₁	27.76±2.12b	12.51±2.56c	3.31±1.74a	0.95a	100%
T ₂	40.63±1.43a	13.37±3.18b	2.93±0.85b	1.27b	100%
T ₃	34.85±1.61ab	13.81±1.12b	3.87±1.07a	0.91a	100%
T ₀	42.34±1.21a	15.50±1.25a	2.43±1.37b	1.29b	100%

Means with similar letters in a column are statistically non-significant.

A comparison of the feed conversion ratios (FCR) of all the four treatment diets prepared during present study revealed that FCR for T₁ was 3.31±1.74, T₂ 2.93±0.85, T₃ 3.87±1.07 while T₀ diets was 2.43±1.37. Better FCR values were recorded for T₀ diets during present analysis and the reason might be the appropriate nutritional values of fishmeal which is required for the growth of *Labeo rohita* (Jatomea *et al.*, 2002; Dumas *et al.*, 2010).

Fish Growth parameters: The FCR values for T₂ diets are almost similar to that of T₀ diets indicating that the diets with 75% acid silage and 25% rice bran and soybean meal can be safely used while preparing aqua feeds for *Labeo rohita* grow outs. However, sole fish silage if used as diets is detrimental for fish growth. Our findings are in line with the results of Goddard and Al-Yahyai (2001) who described that fishmeal can be successfully replaced by fish silage in aqua diets without

affecting the growth and feed utilization parameters (Soltan *et al.*, 2008). Salim and Sheri (1999) reported that *L. rohita* starts proper feeding on commercial feeds at grow out stage and its growth is affected by the protein content of the diets and their feeding frequencies.

Water quality parameters: The physico-chemical parameters recorded during present study are mentioned in table 3. Average water temperature for all the treatment aquaria was 32.03±0.20, average dissolved oxygen was 5.37±0.26, pH 7.34±0.47, salinity 1.16±0.13 and total dissolved solids were recoded as 1100.26±36.81. Lawson (1995) documented that for optimum fish yield water temperature, dissolved oxygen, transparency, pH and salinity must be kept at optimal level compatible with fish species. All these parameters were with in permissible limits for *Labeo rohita* growth (Kamal *et al.*, 2007).

Table 3: Water quality parameters during 90 days of feeding trial. Means with similar letters in a column are statistically non-significant.

Treatments	Temperature (°C)	DO (mgL ⁻¹)	pH	Salinity (mgL ⁻¹)	TDS (mgL ⁻¹)
T ₁	31.83±0.48a	5.45±0.16a	7.17±0.35a	1.06±0.9a	1088.33±40.91a
T ₂	32.07±0.58a	5.03±0.63a	7.10±0.81a	1.10±0.20a	1104.67±32.12a
T ₃	31.94±0.33a	5.66±0.31a	7.06±0.25a	1.13±0.25aa	1148.04±65.58a
T ₀	32.31±0.57a	5.36±0.26a	8.06±0.75a	1.36±0.15a	1060±55.23a
Average	32.03±0.20	5.37±0.26	7.34±0.47	1.16±0.13	1100.26±36.81

At the end of feeding trial, the proximate analysis revealed that non-significant differences in moisture

contents were recorded for all the treatment and control diets. However, significantly lower protein contents were

observed in fish fed with T₂ and T₃ treatment diets. Similarly, significantly lower fat contents were observed in fish fed with T₁ diets while significantly higher ash contents were recorded in fish fed with T₂ diets (table 4). Our findings are in line with findings of Gumus *et al.* (2009) and Majumdar *et al.* (2014).

Hematological indices: The hematological indices of *Labeo rohita* grow out recorded during present study are mentioned in table 5. Statistical analysis for RBCs indicated significant differences among T₂, T₃ and T₀

diets. Similarly, significant differences in WBCs and platelets were observed in all the treatment and control diets while significantly higher hemoglobin contents were recorded in fish fed with T₁ diets. Values for growth hormones in fish fed with T₁, T₂, T₃ and T₀ diets was recorded as 2.34±0.01 ng/ml, 2.34±0.01 ng/ml, 2.27±0.01 ng/ml and 2.18±0.01 ng/ml, respectively. Hormones T₃ and T₄ showed significant differences for all the treatment and control diets.

Table 4. Proximate analysis of fingerling *Labeo rohita* after 90 days of feeding trail. Means with similar letters in a row are statistically non-significant.

Parameters	T ₁	T ₂	T ₃	T ₀
Moisture	4.98±0.43a	4.86±0.37a	5.28±0.69a	5.68±0.32a
Protein	57.60±2.39bc	62.49±0.86a	54.06±1.33c	60.07±1.29ab
Fats	16.28±0.64b	19.70±0.74a	18.77±1.33a	18.45±0.91a
Ash	10.64±0.55b	12.86±0.47a	10.44±0.91b	11.23±0.75b

Table 5. General hematology of *Labeo rohita* grow outs after feeding on experimental feeds of fermented silage. Means with similar letters in a column are statistically non-significant.

Treatments	Blood Parameters						
	RBC x 10 ¹² /L	WBC x 10 ¹² /L	Platelets x 10 ⁹ /L	Hb g/L	Gh ng/ml	T ₃ µg/dl	T ₄ µg/dl
T ₁	1.57±0.01 ^b	19.33±0.03 ^a	5.37±0.02 ^c	6.26±0.1 ^a	2.18±0.01 ^c	0.27±0.01 ^d	0.60±0.02 ^c
T ₂	1.66±0.03 ^a	18.56±0.01 ^c	6.82±0.01 ^a	5.57±0.41 ^b	2.31±0.01 ^a	0.90±0.02 ^a	0.71±0.02 ^a
T ₃	1.57±0.02 ^b	18.15±0.02 ^d	4.22±0.02 ^d	5.60±0.20 ^b	2.27±0.01 ^b	0.96±0.03 ^a	0.76±0.01 ^b
T ₀	1.37±0.02 ^c	18.63±0.02 ^b	6.63±0.02 ^b	5.80±0.11 ^b	2.34±0.01 ^a	0.81±0.02 ^c	0.92±0.04 ^a

Our results are in line with the findings of Nasir and Al-Sraji (2013) who investigated the effects of different levels of dietary protein and fats ration on some blood parameters in common carp fingerlings reared in cage culture and concluded that dietary protein levels of 23.68% had Hb, Hct, RBC and WBC counts higher as compared to lower protein level of 13.82%. They concluded that in order to prevent any adverse effects on fish hematology, diets containing lower than 23% dietary protein should be avoided. It can be therefore concluded that the replacement of fishmeal by fish silage in ratios 75% silage and 25 % rice bran and soybean meal having CP levels of 35% are safe for fish health.

Histology: Histological examination of normal fish liver comprises strands of hepatic cells, which are hexagonal in shape and large in size with centrally located nuclei and homogenous cytoplasm around the central vein. Although the treatment diets showed normal histopathology without any alteration however, the liver

of the fish fed with T₁ diets showed some inflammation of tubules and necrosis of hepatocytes (figure 1). A possible reason of these alterations in fish fed with 100% silage may be due to acidic retentions found in pure acid silage. Our findings are comparable with the results of Shaw and Handy (2006) who reported retarded growth and nutritional uptake in fish and both these are associated with alteration in liver pathology.

Kidneys of fish in normal conditions are composed of numerous renal corpuscles, renal tubules and well developed glomeruli (Das and Mukerjee 2000). During present study, even the sole acid silage (T₁) did not affect kidney tubules and renal corpuscles (figure 2). Hall and Bellwood (1995) reported that intestinal mucosa appears to be dynamic and highly responsive to food availability. During present study, normal intestinal villi were observed for all the fish fed with treatment and control diets (figure 3).

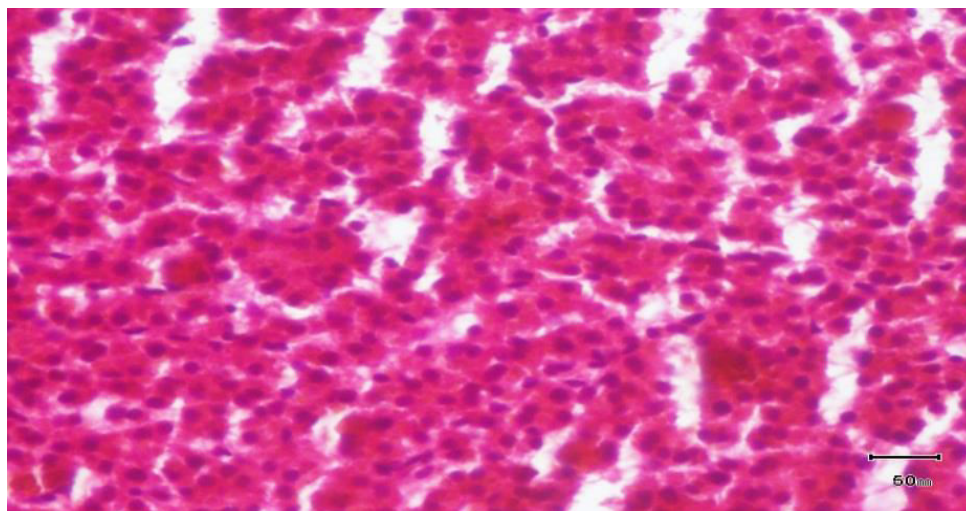


Figure 1. Liver 100% fish silage: Necrosis of hepatocyte and inflammation of tubules. (H&E; 40 X).

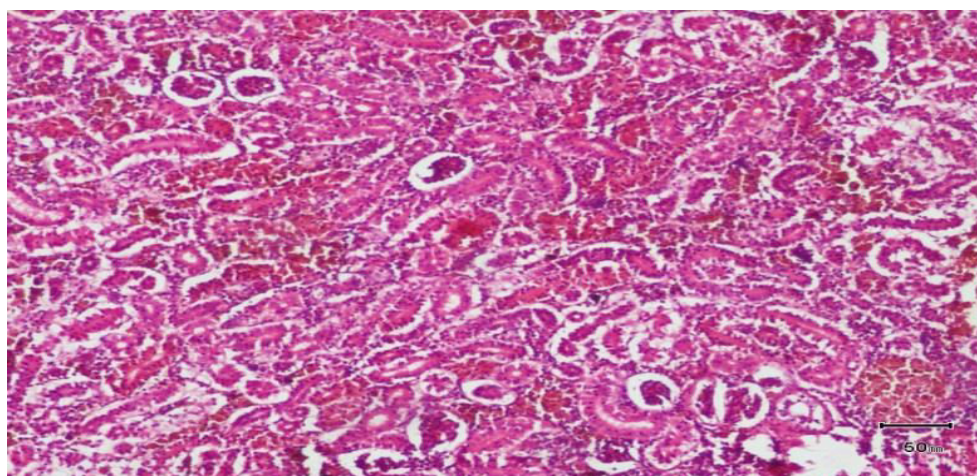


Figure 2. Kidney, 100% fish silage: kidney tubules and renal corpuscles are normal. In some kidney tubules pinkish clot is present. (H&E; 100 X).

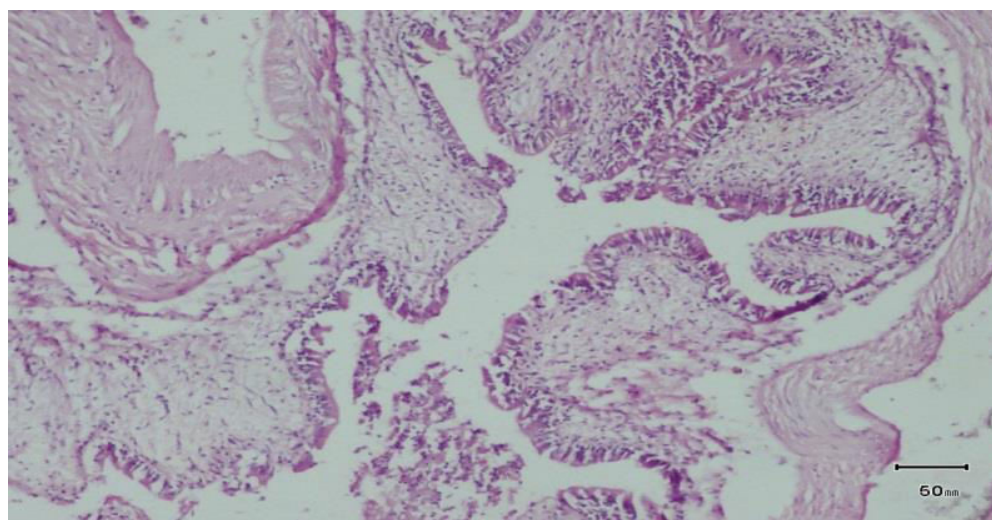


Figure 3. Intestine, 100% fish silage. Normal intestinal villi, the lumen has long epithelial cells. The villi are little elongated. (H&E; 40 X).

Conclusions: It can be concluded from present study that fish acid silage is safe and can be successfully used in preparation of aqua feeds for *Labeo rohita*.

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