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Original Research Article

Poultry Visceral Ensilage as A Replacement Ingredient in Fish Feed and Its Effect on Growth, Survival, Gut Histology and Microbiota in Fry of Common Carp (*Cyprinus carpio* L.)

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ABSTRACT:
Incorporating poultry waste in fish feed could reduce the price of fish feed considerably. The study was conducted to test the efficacy of chicken visceral silage as a replacement of fish meal in fish feed formulations. Broiler offal was procured from several slaughter houses and was ground and preserved with acid mixture (sulphuric and formic acid). The fluid ensilage was neutralized and sun dried to obtain dry silage. Common carp fry (average weight 0.71g) were used as experimental fishes for the study. 5 kinds of feeds were formulated for the experiment at inclusion levels of 0, 5, 10, 15 and 20% of visceral silage replacing equal amounts of fish meal. Triplicate tanks were set up for each group of formulated feed. Feeding trials were conducted for a period of 60 days. At the end growth parameters and histopathological studies were done. The growth parameters suggested significant increase in Weight Gain and SGR ($p>0.05$) in treatment T4 (20% inclusion of visceral silage).Also, comparative study of histopathology of intestine and liver showed no major diseased conditions. Thus, it concludes that broiler visceral silage could be a potential replacement of fish meal in feed formulations.

Keywords: Poultry waste management, Offal silage, Fish meal replacer, Broiler visceral silage, Economic fish feed

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INTRODUCTION

Majority of fishery in India is from Inland Fisheries, predominantly from aquaculture of which freshwater aquaculture accounts for about 95% (Jayasankar, 2018). Carp culture is the backbone of Indian freshwater aquaculture (NABARD, 2018).Fish nutrition is an important aspect of aquaculture. Optimum fish feed in intensive or semi-intensive type of aquaculture accounts for 40-60% of total expenditure. As a source for proteins and lipids dependency on these fish products is about 70% for carnivorous fish and 50% for shrimps (Tacon, A.G., 1995). When it comes to production of commercial fish feed with optimum levels of dietary requirements it becomes quite expensive because most of the protein in fish feed comes from fish meal which is costly. Again, at places far from sea coast availability of fish meal is an issue. Thus, there is a great need to produce cheap fish feed to maximize the earnings of farmers. One alternative which fish farmers use is to make fish feed only from plant sources but these lack essential amino and fatty acids (Tacon, A. G., 1995). Other less used alternatives are animal by products. These resources if available in plenty can reduce the cost of feed considerably. So, it's important that adequate research is done on these alternatives for suitable feed formulation. A fine solution to these problems comes from utilization of wastes from meat industries. Such wastes can be from fish, poultry or other slaughter houses. This paper discusses about silage production and its incorporation in fish feed.

Silage is wet fodder when preserved either in an acidic medium directly or by using bacteria which produces lactic acid in anaerobic condition so as to be used as animal feed in future. The formation of silage is done in airtight containers or pits called a silo. There are basically two types of silage. Acid preserved silage uses acids to lower the pH to an extent such that no growth of spoilage bacteria, insects or fungus takes place. The enzymes present naturally in the substance along with the acid subsequently degrades the minced substance into a liquid. In Fermented silage method, a carbohydrates source known as accelerator and a starter culture of suitable bacteria called the inoculum is provided for fermentation (Bakrie, 2017). The use of poultry byproducts such as chicken feathers, heads and feet to produce silage requires fermentation methods with suitable inoculums (Bakrie, 2017; Rachmawati, 2019). This is not possible with a farmer without use of sophisticated equipment. But visceral silage preserved with acids can be produced locally by farmers quite easily. In the present research, silage was produced using broiler viscera as it is available in plenty. Again, it could be an efficient way of poultry waste management.

MATERIALS AND METHODS

Common carp fry, *C.carpio* (0.71±0.01) were obtained from District Fisheries Office, Gajapati, Odisha, India. The fishes were disinfected using 5ppm $KMnO_4$ solution for 15 mins. The dead and unhealthy fishes were separated. Then they were redistributed to 15 HDPE lined well aerated circular tanks of 80 liters each. Each tank contained 20 fishes. The fishes were acclimatized to the conditions for a week. Chicken viscera was collected from several slaughter houses. A part of the procured viscera was boiled. This released the lipid content which floats over the surface of water. The water was decanted and the boiled viscera was mixed with raw viscera in the ratio 2:3. It was minced with a grinder. Acid mixture (5% w/w) i.e Formic (85%) and Sulphuricacid (98%) was added in the ratio 3:2. Ascorbic acid at rate of 1.5/kg was added as an antioxidant to prevent rancidity. The viscera was left for 10 days in a closed glass jar. The viscera gradually gained light brown coloration, pungent smell and fluidity. This indicated the formation of ensilage. The pH and physical characteristics was monitored daily. pH was kept at 2.5±0.1 during the ensilation process.

The formed ensilage was neutralized with $NaHCO_3$, sun dried and a proximate analysis was done as per AOAC, 2005, with minor modifications. The ensilage showed a high protein % of 55.05±0.10. The lipid% was found

to be 25.67±0.44 while ash % was 18.15±0.44. The values have been summarized in Table 1.

Table 1: Proximate Composition of Silage

Nutrient Profile	Value
Dry Matter	86.11±0.35
Crude Protein	55.05±0.10
Crude Lipid	25.67±0.44
Ash	18.15±0.44

It was then incorporated in fish feed as per nutritional requirements recommended by Food and Agriculture Organization of the United Nations (FAO), to obtain iso-nitrogenous, iso-lipid and iso caloric diets. The values of inclusion level in percentage along with their proximate compositions have been summarized in Table 2.

Table 2: Feed formulation of Experimental diets

Ingredients	**% Composition**				
	C	**T1**	**T2**	**T3**	**T4**
Silage	**0**	**5**	**10**	**15**	**20**
Fish Meal	30	25	20	15	10
GNOC*	38	40	40	42	44
Rice Bran	18	17	17	16	14
Sunflower Oil	2	2	1	1	1
Wheat Flour	9	8	9	8	8
BHT*	1	1	1	1	1
Premix*	1	1	1	1	1
CMC*	1	1	1	1	1
Total	**100**	**100**	**100**	**100**	**100**
Proximate Analysis (%)					
Dry Matter	92.52±0.27	92.29±0.15	92.08±0.57	91.81±0.57	91.76±0.63
Crude Protein	41.64±1.03	41.45±0.93	41.52±1.22	41.38±0.84	41.42±0.55
Crude Lipid	10.21±0.79	10.37±0.47	10.87±0.23	11.01±0.25	11.35±0.68
Ash	12.77±0.85	12.65±0.62	12.12±0.07	12.07±0.45	12.05±0.82
Carbohydrates	35.12±1.05	35.52±1.06	35.50±1.20	35.54±1.17	35.18±1.18
Energy(kcal/100g)	386.11±7.43	386.75±5.00	390.90±1.99	391.58±2.75	393.27±3.38

*__GNOC__– Ground Nut Oil Cake; **BHT**-Butyrated Hydroxy Toluene; **Premix**– Vitamin and Mineral Premix (Agromin Forte); **CMC**- Carboxy Methyl Cellulose

Feeding trials were conducted for a period of 60 days. The weight of fishes was recorded every 15 days. Water quality parameters i.e. pH, D.O and temperature was monitored every 2-3 days and maintained. Temperature varied at 29±1°C. Daily water exchange of 25% kept the pH level at 7.2±0.3. The D.O value was 9.2±0.5 mg/L.

The various growth parameters were calculated by the following equations.

Weight Gain (g) = $Final\ Body\ Weight(g) - Initial\ Body\ Weight(g)$

Daily weight gain (g) = $\frac{Weight\ Gain(g)}{No.of\ Days}$

$$\textit{Weight gain (\%)} = \frac{Weight\ Gain(g)}{Initial\ Body\ Weight\ (g)} X100$$

$$\textit{Specific Growth Rate (SGR)} = \frac{ln(Final\ Body\ Weight\ (g)) - ln(Initial\ Body\ Weight\ (g))}{No.\ of\ Days} X100$$

$$\textit{Feed Conversion Ratio (FCR)} = \frac{Total\ feed\ intake\ (g)/fish_{*}}{Body\ Weight\ Gain(g)}$$

$$\textit{Protein Efficiency Ratio (PER)} = \frac{Weight\ Gain\ (g)}{Total\ Protein\ Intake\ (g)/fish} X100^{**}$$

$$\textit{Survivality (\%)} = \frac{Total\ fishes\ a\ live\ at\ the\ end\ of\ experiment}{Initialno.of fishes} X100$$

* *Total feed Intake (g)/fis h= 4% of Fish Body Weight X No. of Days*

** *Total Protein Intake (g)/fish =* $Total\ feed\ Intake\ (g)/fish\ X\ Protein\ \%\ of\ diet$

Histological analysis was done for intestine and liver tissues. The paraffin sectioning method was followed for the process (Slaoui & Fiette, 2011). After dissection the tissues were made into paraffin blocks and sectioned at 7µm by a rotary microtome and stained with hematoxylin-eosin (HE) stain. The stained tissues were embedded over slides and observed under a stereoscope.

The ratio of the weight of the liver and intestine in relation to the total body weight of fish was calculated separately from the following organsomatic index formula according to Adesina, 2017.

$$\textit{Hepatosomatic Index (HSI)} = \frac{Weight\ of\ the\ liver}{Total\ weight\ of\ the\ fish} X100$$

$$\textit{Viscerosomatic Index (VSI)} = \frac{Weight\ of\ the\ viscera}{Total\ weight\ of\ the\ fish} X100$$

Gut microbial analysis was done by randomly selecting fishes from each treatment. The fishes were starved for 24hrs. and were dissected after being cleansed with alcohol (70%). A fine paste of the gut dissected was prepared by mixing it with saline solution (0.85%). This mixture was further diluted and spread in sterilized nutrient agar medium followed by incubation of 24 hrs. and 48 hrs. respectively to count the total bacterial colony formed. (Heikkinen et al., 2006)

Statistical Analysis was done with SPSS, version 11.One way ANOVA was used to compare means (p=0.05).The differences between groups was found out by Duncan's Multiple Range Test (DMRT). Significance of differences was defined at $p<0.05$.

RESULTS

Table 3 represents the various parameters depicting the growth performance. The Weight Gain value varied from 3.69±0.02 (Treatment C) to 4.04±0.05 (Treatment T4).Treatment C and T1 showed similar weight gain ($p>0.05$).I t showed slight increase ($p<0.05$) in T2 and T3 which were again similar and was the highest in T4.The value of Weight Gain % was lowest in C i.e 514.32±10.55.The weight gain percentage was equivalent in C, T1 and T2.There was a significant increase in T3 as compared to C and T1 but no significant difference was noted when compared to T2.The highest value was seen in T4 i.e 566.39±15.62 but that was not significantly different from T3. Daily Weight Gain was similar to weight gain with the highest value being 0.0673±0.0008 in T4 and lowest value equal to 0.0615±0.0003 in C. The values of C and T1 did not show any significant difference

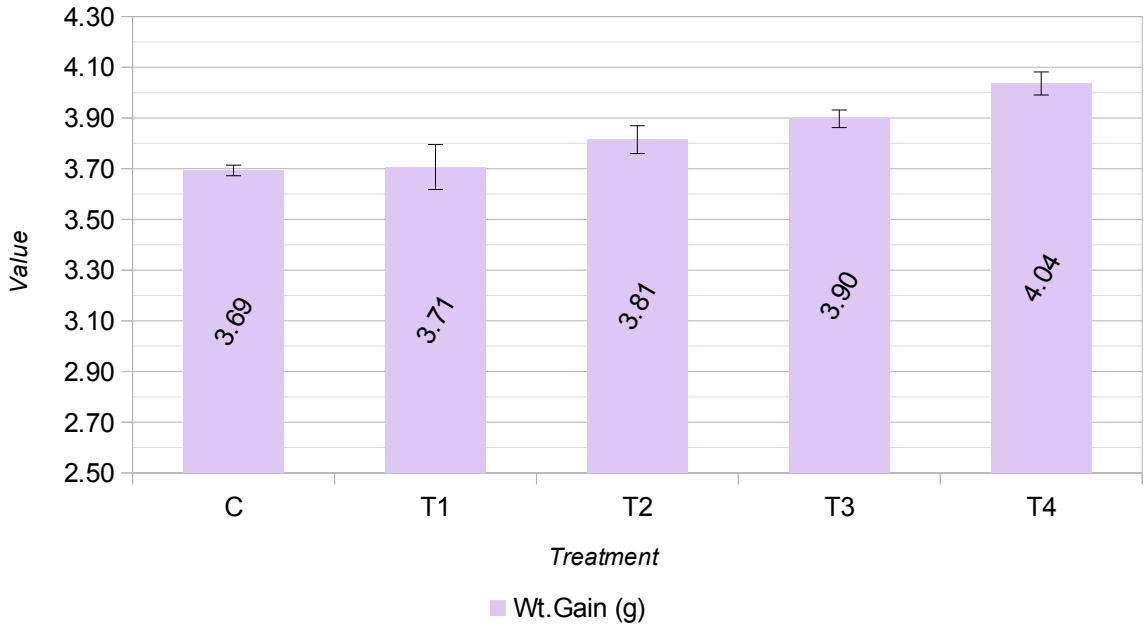
between groups and so did T2 and T3.SGR varied between 3.03±0.03 in C and 3.16±0.04 in T4.Significant difference was not seen between groups C, T1 and T2.But T3 showed significant increase as compared to C and T1, although there was no significant difference compared to T2. The values of FCR, PER and Survival % did not show any significant difference upon statistical analysis ($p>0.05$). The lowest value of FCR was noted in T4.It varied between 2.136±0.023 in T4 and 2.158±0.023 in T1. PER ranged from the highest value 1.130±0.012 in T4 to lowest value equal to 1.116±0.007 in C. Survival % was around 90% in all the groups and did not show any significant difference in any of the groups.

Table 3: Growth performance of fry of *Cyprinus carpio* fed with experimental diets

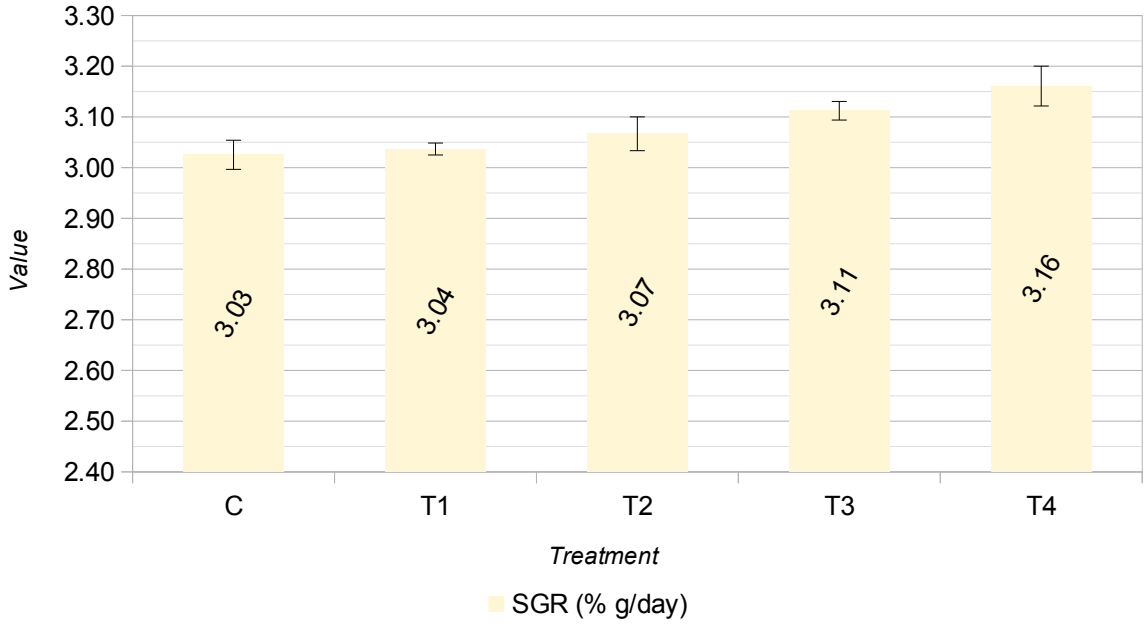
Growth Parameters	**Experimental Feed Groups**				
	C	T1	T2	T3	T4
Initial Wt.(g)	0.72±0.01	0.71±0.01	0.72±0.01	0.71±0.01	0.71±0.01
Final Wt.(g)	4.41±0.02^{a}	4.42±0.10^{a}	4.53±0.05^{b}	4.61±0.05^{b}	4.75±0.04^{c}
Weight Gain(g)	3.69±0.02^{a}	3.71±0.09^{a}	3.81±0.05^{b}	3.90±0.03^{b}	4.04±0.05^{c}
Weight Gain (%)	514.32±10.55^{a}	518.48±4.37^{a}	529.80±12.57^{ab}	547.13±7.10^{bc}	566.39±15.62^{c}
Daily Weight Gain(g)	0.0615±0.0003^{a}	0.0618±0.0015^{a}	0.0636±0.0009^{b}	0.0649±0.0006^{b}	0.0673±0.0008^{c}
SGR (%g/day)	3.03±0.03^{a}	3.04±0.01^{a}	3.07±0.03^{ab}	3.11±0.02^{bc}	3.16±0.04^{c}
FCR	2.152±0.013	2.158±0.023	2.146±0.016	2.149±0.013	2.136±0.023
PER	1.116±0.007	1.118±0.012	1.123±0.008	1.124±0.007	1.130±0.012
Survival (%)	91.67±2.89	91.67±5.77	88.33±5.77	90.00±5.00	91.67±2.89

*Values with different superscripts in the same row suggest significant difference ($p<0.05$).

It is clear from the results that Treatment C and T1 overlap in weight ,while rest of the treatments shows a gradual increase with increase in silage inclusion percentage in the order T2,T3 and T4.On finding out the weight gain in interval of 15 days, it can be noticed that higher weight gain upto 30 days in T3 and T4 as compared to C,T1 and T2 while weight gain was similar in the next 30 days in each of the treatments .The increased weight gain and SGR in each of the treatments are depicted in Graphs II(A) and II(B)



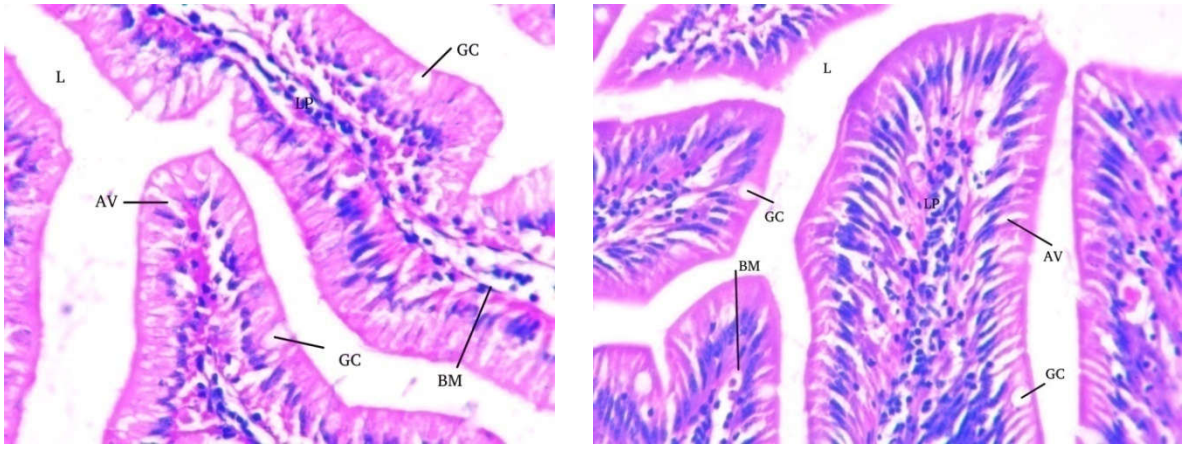
(A)



(B)

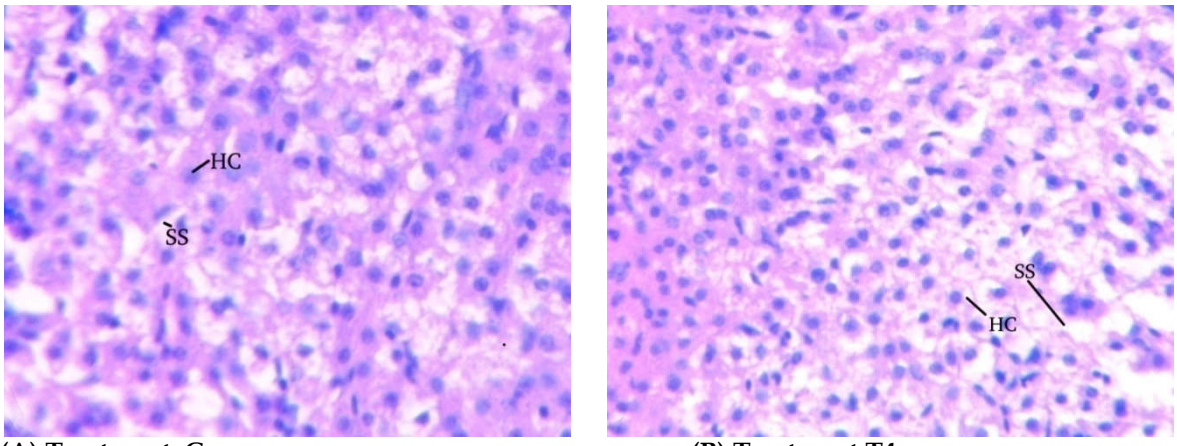
Graph I: (A) Mean Weight Gain and (B) Mean SGR across the treatments. ($p>0.05$)
The Std. Dev. has been represented by Y -error bars

Fig 1(A) and (B) show sections of intestine of fish of treatments C and T4 respectively. In both cases long villi and normal distribution of goblet cells was seen. The brush border and mucosa was intact. No necrosis was noticed. Fig 2(A) and (B) show section of liver of treatments C and T4 respectively. In any of the treatments no fat droplets were noticed. No hyperplasia or hyperemia was noticed. Nuclei was centric and few melanomacrophage centers could be noticed.



(A) Treatment C **(B) Treatment T4**

Figure 1: Intestine of treatments C (A) and T4(B)
GC-Goblet Cell, **BM**- Basement Membrane, **LP**-Lamina Propria, **AV**-Absorptive Vacuole, **L**-Lumen



(A) Treatment C **(B) Treatment T4**

Figure 2: Liver of treatments C (A) and T4(B)
HC-Hepatocyte, **SS**-Sinusoid

The gut microbial experiment conducted for the treatments (C, T1, T2, T3 and T4) showed significantdifferences in the results. The CFU count of the control was found to be the highest with 4.126±0.0106 log CFU/ml. Following it the second highest microbial count was observed to be 3.607±0.196 log CFU/mlfor T1. T4 with the 20% inclusion of the silage in fish feed showed the lowest CFU count of 2.516±0.0601 log CFU/ml, despite the count being similar to that of T2 and T 3 with microbial count 2.838±0.152 log CFU/ml and 2.548±0.122 log CFU/mlrespectively. The values observed have been represented in the following Table 4 and Graph II. The hepatosomatic and viscerosomatic index were found out as mentioned in the Table 5 below.

Table 4: Microbial colonies observed in the gut of *C.carpio* fry fed with experimental diets

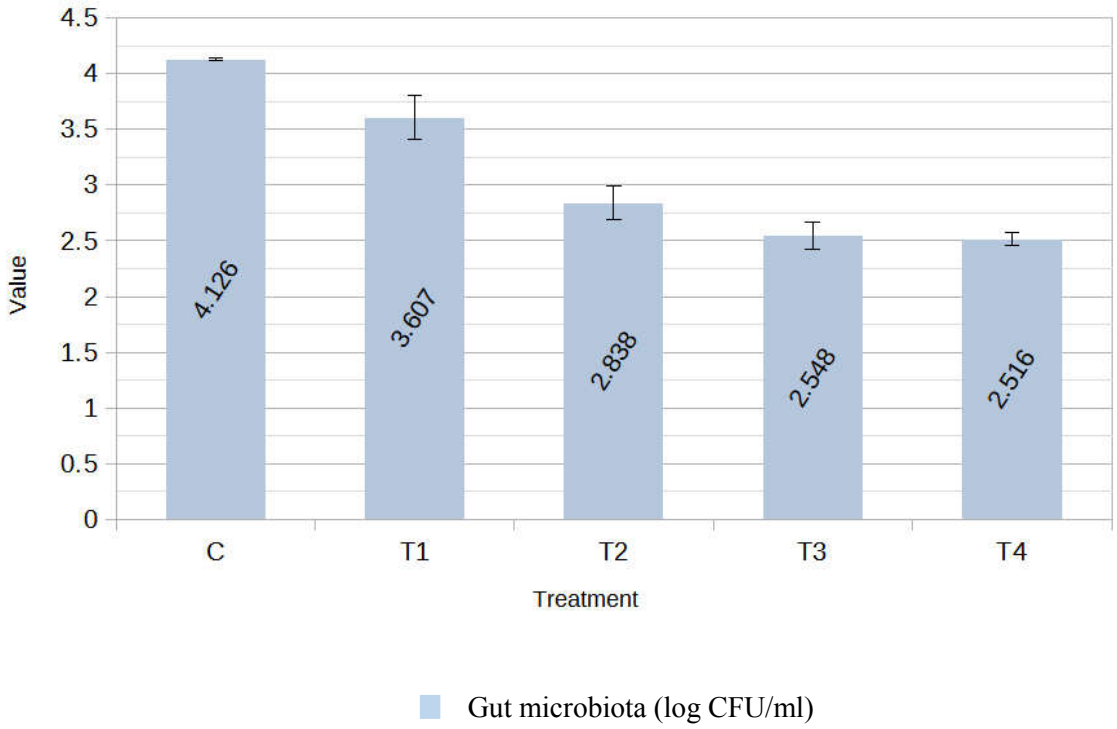
Treatments	Microbial Count (log CFU/ml)
C	4.126±0.010[a]
T1	3.607±0.196[b]
T2	2.838±0.152[c]
T3	2.548±0.122[d]
T4	2.516±0.060[d]

*Values with different superscripts in the same column suggest significant difference ($p<0.05$).

Table 5: HSI and VSI of fry in various treatments.

Treatments	HSI	VSI
C	0.393±0.033	2.184±0.160
T1	0.399±0.026	2.201±0.030
T2	0.411±0.029	2.213±0.228
T3	0.412±0.026	2.219±0.176
T4	0.421±0.022	2.225±0.084

*No significant difference for both HSI and VSI between treatments ($p>0.05$)



Graph II: Gut microbiota of *C. carpio* fry across various treatments.

DISCUSSION

The study showed that fishes fed with diets with maximum inclusion of silage had enhanced growth and improved feed utilization in Common carp. As seen the highest growth was seen in treatments T3 and T4. This could be due to better digestibility of the feed due to high inclusion of silage in T3 and T4. The proteolytic reactions that takes place during formation of silage breaks down proteins into short chains and free amino acids resulting in easy digestibility. The enhanced growth suggested that silage as an ingredient in feed is optimum for nutrition of fish. However, the weight gain was not significant enough to result in significant changes in FCR and PER. The said values could have shown substantial difference

if the experiment was continued for a longer duration. The results agreed with Belal et. al., 1995 who used chicken visceral silage as feed in *Oreochromis niloticus,* finding out that 20% inclusion of silage in fish feed did not show any negative impact on growth of the fingerlings. T hough they could not use visceral silage greater than 20 % due to high lipid content (33%), in the present study the use of defatting technique has enabled us to do trials involving higher inclusion in future.

Chicken viscera was found to have a high fat content and so was the silage produced from it .This needed defatting before inclusion in feed .Fagbenro and Fasakin,1996 found around 42% fat in silage produced and hence used the dry rendering method for the purpose .The value of fat content in untreated viscera silage agrees with the results in the current study. They suggested that inclusion to about 40% did not decrease the growth rate in catfish, *Clarias gariepinus* fingerlings. However this may have been possible due to use of citric acid (organic acid) for ensiling. Since we used mixture of sulphuric acid and formic acid we considered inclusion of 20% to be a safe level. But the positive results indicate that higher level of silage could be studied as well.

In sections of intestine long villi suggested good absorptive surface area. Since diets had lipid levels not exceeding optimum limits hence no fatty deposition was noticed. The intestines and livers of both the treatments were normal. Although much work has not been done referring to histological changes in gut due to chicken silage. Jasim et.al, 2016 also reported no changes in histology due to feed with fish silage which agrees to the findings in our current study.

The gastro intestinal microflora plays an important role as one of the growth parameters. It has been found to have profound effects on the anatomy, physiology and immune system of the host organism. The gut microflora experiment is often conducted to detect the presence of any beneficial bacteria in the GI tract of the host. Moreover, it is also conducted to detect the disease-causing pathogens in the host's body. The results of the above experiment conducted, showed similarities with research performed by Goosen et al., 2014. The results showed a gradual decrease in the microbial count in the gut of the fishes fed with the experimental diet as compared to that of control diet. This declination in gut microbes and increased fish growth rate is observed due to the presence of formic acid in the silage. Being a short chained organic acid, formic acid poses antimicrobial properties. Studies have proved that these acids in the feed lowers the bacterial count thus increasing the feed hygiene while also decreasing the competition for nutrients in GI tract between the host animal and microflora by killing the pathogenic bacteria. Although being lowest in the microbial count T4 showed the highest fish growth rate, hence, it can be concluded that the fishes fed upon with the T4 diet consisted of certain beneficial microbes which resulted in greater and healthier growth rate of the fishes.

CONCLUSION

It infers from the results that feed with about 15-20% inclusion poultry visceral silage is good for nutrition of juvenile fish without showing any adverse effects on growth parameters as well as histology of intestine and liver. The present method of silage production was by using mixture of Sulphuric and Formic acid. The production could be tried with Acetic acid (commercially available vinegar) as it is easily available and neutralised. There can be further research on inclusion levels. Feeding trials can be done with complete replacement of fish meal. Research could be done on hematological parameters as well stress level indicators such as glucose and cortisol.

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