



# Effects of fish waste silage and vitamin-C supplemented diet on growth and haematological parameters of common carp, *Cyprinus carpio* (Linnaeus, 1758) fingerlings

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## Abstract

A 60-day experiment was conducted to evaluate the growth performance, feed utilization and haematological parameters of common carp, *Cyprinus carpio* under four dietary treatments with three replicates for each. These were control (0%), T1 (15%), T2 (30%) and T3 (45%) fish waste silage containing diets. Nutritional profile of experimental feeds was: crude protein (25–25.75%), lipid (8.46–8.96%), moisture (10.64–11.16%), ash (14.05–14.87%), crude fiber (4.07–4.32%), and nitrogen-free extract (35.69–37%). The haematological parameters were observed of *C. carpio*, which improving White blood cells, Red blood cells, haemoglobin (Hb) and Hematocrit (HCT) within the healthy physiological range. Growth performance parameters were observed at the end of the experiment. The highest length gain ( $5.96 \pm 0.05$  cm), weight gain ( $8.43 \pm 0.15$  g), percentage weight gain ( $161.44 \pm 10.14\%$ ), specific growth rate ( $0.46 \pm 0.01\%$ ), protein efficiency ratio ( $3.03 \pm 0.18$  g), hepatosomatic index ( $0.05 \pm 0.00\%$ ), and intestinal somatic index ( $0.33 \pm 0.00\%$ ) was observed compared to the control groups. The highest feed conversion ratio ( $1.91 \pm 0.47$ ) was observed in control group compared to the treatment group. Carcass composition of the fish at the end of the trial was similarly analyzed for protein (58.87–60.29%), lipid (13.10–13.78%), moisture (10.25–10.96%), ash (9.21–9.32%), and NFE (6.45–0.73%) content. Water quality parameters remained within acceptable limits throughout the study period. The results suggest that specific dietary treatments significantly enhanced growth, haematological parameters, carcass composition, and feed utilization in common carp.

**Keywords:** carcass composition; *Cyprinus carpio*; growth; haematology; silage; water quality

## 1 | INTRODUCTION

One important factor in maintaining food security on a worldwide scale is aquaculture, which involves the cultivation of aquatic creatures like fish, shellfish, and aquatic

plants. Aquaculture offers a sustainable way to fulfill the growing need for healthy food, which is driven by the ever-increasing global population (Duarte *et al.* 2022). In areas where other protein sources, like cattle, are scarce,

it makes a big difference to the world's protein production (Sandström *et al.* 2022). An abundance of vitamins, minerals, vital fatty acids, and high-quality protein may be found in fish and other marine creatures. One effective way to produce protein-rich food is through aquaculture, which involves the cultivation of aquatic organisms (Radhakrishnan *et al.* 2020). Reducing dependence on conventional farming and wild fisheries, aquaculture broadens the sources of food production. Food production systems on land and wild fish stocks are both helped by this diversification. It lessens the impact of food shortages and price volatility by offering a different supply of healthy food to communities (Choudhury *et al.* 2022). The only viable alternative that can address these inadequacies and meet the growing demand for high-quality protein is aquaculture. Since feed accounts for over half of the overall operating cost, its high price tag is one of the main obstacles to the growth of inland aquaculture (Llanes *et al.* 2008). One of the most prevalent feed constituents in fish feeds, fishmeal, satisfies the protein needs of fish. However, fishmeal can be quite pricey and its availability might be unpredictable. Hence, dietitians are on the lookout for alternatives to fishmeal that are both affordable and of high quality for use in fish feed (Santana-Delgado *et al.* 2008).

The valorization of food waste is essential for achieving the United Nations' Sustainable Development Goals aimed at attaining zero waste by 2030, which encompasses economic, social, and environmental dimensions of sustainable development (United Nations 2019). Consequently, the recycling of food waste has emerged as a significant issue that requires attention. Fish are a nutritious dietary source and account for around 17% of global animal protein consumption. Nonetheless, its bodily constituents vary according to species, sex, age, and season (Obiero *et al.* 2019). In 2018, the aggregate fish production from aquaculture and wild catch was around 178 million tonnes, exhibiting a growth rate of 2–3.5% (FAO 2020). By-products from fish processing constitute roughly 60 – 70% of the live weight of most commercially processed fish (Ananey-Obiri *et al.* 2018). The majority of fish processing waste is disposed of in landfills and/or aquatic environments, leading to environmental contamination and economic detriment. Fish byproducts encompass the heads, viscera, fins, scales, bones, and trimmings, which may contain substantial levels of nutrients like proteins, lipids, minerals, vitamins, bioactive compounds, and enzymes (Villamil *et al.* 2017). The internal organs of fish, such as the intestine, liver, and swim bladder, comprise almost 30% of their entire body weight. These are common by-products of fish processing that are not employed for human consumption and are dumped as 'wastes,' hence contributing to environmental pollution upon disposal (Haider *et al.* 2016). Fish waste has diverse applications in food and biotechnology owing to its advanta-

geous functional and chemical qualities (Nawaz *et al.* 2020).

The definition of fish silage is a liquid product made from fish or fish parts that has been treated with acids, enzymes, or bacteria that produce lactic acid. The mass is then liquefied by the fish's own enzymes (FAO 2007). As a dark liquid, fish silage is both easily digestible and a great protein source. Nutritional value and varied production methods are hallmarks of silage (Tatterson and Windsor 1974). A number of benefits can be achieved by producing fish silage from byproducts of fish processing, such as lowering feed costs, doing away with the need to store fresh fish feed, and creating feed that is both easily digestible and nutritionally comparable to natural feed (Tatterson and Windsor 1982). Because fish-silage is a liquid, it needs to have its water content lowered for it to be used optimally in animal diets.

One of the most well-known freshwater fish species, the common carp (*Cyprinus carpio*) originated in Asia and Europe. One of the most dispersed freshwater fish species, this one flourishes in eutrophic environments, as is typical of lakes and big rivers, and it has been imported all over the world. Although it is widely considered an invasive species because of its ability to displace native fish populations and disturb aquatic ecosystems, its main reasons for introduction to different locations include aquaculture, recreational fishing, and human consumption (Badiou *et al.* 2011). Common carp have strong, lengthy bodies and can have a yellowish belly and a variety of back colors, including silver, olive-green, or brass. To help them find food in the sand, they have two sets of barbels close to their lips. Their bodies are covered in huge, plate-like scales, and they feature a lengthy, serrated dorsal fin. Under ideal circumstances, an adult common carp can weigh up to 40 kg (88 lb) and measure 45 to 120 cm (16 to 47 in) in length (Koehn *et al.* 2000). The benthic diet of common carp consists of small invertebrates and plant tubers that the fish digs up with its barbels. As a distinctive feeding behavior, they draw in nutrient-rich muck and silt and then release the excess sediment, which can make water more murky (Sibbing *et al.* 1986). The objective of the present study to evaluate the effects of silage-based diets on growth performance, haematological parameters, carcass composition, and water quality parameters of common carp (*C. carpio*).

## 2 | METHODOLOGY

### 2.1 Location of the work

The research work was carried out at the Live Fish Laboratory Department of Aquaculture, L.S.P.N. College of Fisheries, Kawardha, Chhattisgarh.

### 2.2 Experimental fish

The experimental fish were collected from the local state-owned private hatcheries at Pondi, Kabirdham (C.G.), In-

dia.

### 2.3 Feed formulation

The raw fish byproducts, including heads, viscera, fins, scales, bones, and trimmings, will be collected at the local fish market in Kawardha to prepare fish silage. After proximate analysis, three diets having 25% protein were prepared containing (1) 0.5 g fish silage, (2) 1 g fish silage and (3) 1.5 g fish silage and were labelled as T1, T2, T3. Diet without silage served as control, which contained fish-meal instead of silage (Table 1). Soybean meal and rice bran were added with silage to maintain the protein level at 25% with different ratios (Table 1). The pH of silage was increased by the addition of calcium carbonate to obviate any damage to fish during intake of feed and subsequent digestion.

**TABLE 1** Ingredients composition of different experimental diets.

Ingredients	Diets			
	Control	T1	T2	T3
Rice bran (g)	38	38	38	38
Mustard oil cake (g)	30	30	30	30
Soyabean meal (g)	20	20	20	20
Fish silage (g)	0	0.5	1	1.5
Vitamin-C (mg)	1	1	1	1
Wheat bran (g)	10	9.5	9	8.5
Mineral mixture (g)	1	1	1	1
Grand total (g)	100	100	100	100

T1, diet containing 0.5 g fish silage; T2, diet containing 1 g fish silage; T3, diet containing 1.5 g fish silage; control, diet without silage.

### 2.4 Experimental design

One hundred twenty fingerlings of *C. carpio*, average weight ranging from 4–5 g will be randomly stocked in four different experimental groups such as T0, T1, T2 and T3 with three replicates, following a completely randomized design (CRD).

### 2.5 Feeding trial

All the diets were prepared and offered to fish in pellet form. Common carp fingerlings showing average weight  $2.34 \pm 0.05$  g and average length  $7.6 \pm 0.03$  cm were collected from the fish nursery at Khaibana (Kala), and were acclimatized in fibre glass tanks (12×18×24 cm) for 5 days then were divided into four groups; three groups received experimental diets and the fourth group was kept on control diet (without silage). All the dietary treatments including control had three replicates. Fish weight and length were taken at the time of initiation of the experiment, and thereafter, the increase in fish weight and length were noted on a fortnightly basis. All the fish were fed at 5% of their body weight twice a day at 8 am and 4 pm. Before feeding, the leftover feed and faecal matter

were removed from water. At the end of the trial, all the fish were harvested, weighed and measured, and some fishes were dried for the determination of body composition. Water quality parameters like temperature, pH and dissolved oxygen (DO) of aquarium water were monitored on a daily basis.

### 2.6 Growth performance

After 60 days of the experimental period, the weight of every fish in the aquariums was measured separately (Lal *et al.* 2022; 2023a). Growth performance was evaluated using the formulae given below.

$$\text{Body length gain (cm)} = \text{Final average length gain} - \text{Initial average length gain}$$

$$\text{Body weight gain (g)} = \text{Final average weight gain} - \text{Initial average weight gain}$$

$$\text{Percentage weight gain (\%)} = (\text{Final average weight gain} - \text{Initial average weight} / \text{Final average weight gain}) \times 100$$

$$\text{Specific growth rate (SGR, \%)} = [(\ln \text{ final weight} - \ln \text{ initial weight}) / \text{No. of days}] \times 100$$

$$\text{Survivability (\%)} = (\text{Total number of fish harvested} / \text{Total number of fish stocked}) \times 100$$

$$\text{Mortality (\%)} = (\text{Number of fish that died during the experiment} / \text{Total number of fish stocked}) \times 100$$

$$\text{Feed conversion ratio (FCR, g)} = \text{Feed given (g dry weight)} / \text{Body weight gain (g wet weight)}$$

$$\text{Protein efficiency ratio (PER, g)} = \text{Net weight gain} / \text{Protein fed}$$

$$\text{Hepato-somatic index (HSI, g)} = (\text{Liver weight (g)} / \text{Weight of fish (g)}) \times 100''$$

$$\text{Intestinal Somatic Index (ISI, g)} = (\text{Intestine weight (g)} / \text{Weight of fish (g)}) \times 100$$

### 2.7 Haematological parameters

Each fish was anaesthetized with clove oil (Merck, Germany) at the rate of 50 µl of clove oil per liter of water before collecting blood samples from fish. All the 5 fish from each tank were used for blood collection and pooled samples from each tank were used for analysis. Blood was collected from caudal vein of fish by using 1.0 ml hypodermic syringe and 24 gauge needles. The collected blood (approximately 400–500 µL from each fish) was immediately transferred into vials coated with thin layer of EDTA, as anticoagulant (for blood and plasma collection). Vials having EDTA coats were shaking gently in order to prevent haemolysis and clotting of blood. In the present experiment, mainly the blood in collected and kept with EDTA for the study of different parameters. TEC and TLC was done with the help of Schaperclaus *et al.* (1991)

method. PCV was measured by Schaperclaus *et al.* (1991) method with slight modification. The total haemoglobin (Hb) content was estimated by using Sahli Haemometer (Marinefield, Germany) (Lal *et al.* 2025). To calculate the MCV, expressed in femtoliters (fl or 10–15 L), the following formula was used. To calculate the MCHC, expressed as grams of haemoglobin per 100 mL packed cells, the following formula was used (Lewis *et al.* 2001).

### 2.8 Carcass composition of common carp

At the end of the trial, proximate composition of under trial fish were determined following the standard methods of Association of Official Analytical Chemists (2005) for moisture, protein, fat and ash contents, respectively.

### 2.9 Water parameter analysis

The water physio-chemical parameters, viz., water temperature, DO, pH, Turbidity, total dissolved solids, conductivity, alkalinity and hardness of the tank water were estimated and recorded fortnightly prior to sampling by the standard methods (APHA 2005). DO and temperature is measured in early morning around 7 – 8:30 AM. The pH was estimated in a digital pH meter 335 (Systronics). The total alkalinity and hardness of water was measured following the standard methodology of APHA (2005); (Damle *et al.* 2023; Lal *et al.* 2023b).

### 2.10. Statistical analysis

The data obtained were analysed by SAS (1999; statistical package; version 22.0 for Windows). Data obtained from

studies based on completely randomized experimental design were subjected to one-way analysis of variance. Results were considered significant at  $p < 0.05$ . Means of each treatment including control then were compared using Duncan's multiple range test for level of statistical significance among treatments.

## 3 | RESULTS

### 3.1 The proximate composition of feed

The proximate composition of experimental feed are represented in Table 2. The proximate composition of the experimental feed included analyses of crude protein, lipid, moisture, ash, crude fiber, and nitrogen-free extract (NFE). The highest crude protein content ( $25.75 \pm 0.020\%$ ) was observed in the T3 group whereas the lowest value ( $25 \pm 0.020\%$ ) was observed in the control groups. The highest crude lipid ( $8.96 \pm 0.015\%$ ) was observed in the T3 group whereas the lowest value ( $8.46 \pm 0.026\%$ ) was observed in the control groups. The highest moisture content ( $11.16 \pm 0.015\%$ ) was observed in the control group while the lowest ( $10.64 \pm 0.021\%$ ) was observed in the T3 groups. The highest crude ash ( $14.87 \pm 0.02\%$ ) was observed in the T3 group compared to the other treatment groups. The highest crude fibre ( $4.32 \pm 0.015\%$ ) was observed in the control group and whereas the lowest ( $4.07 \pm 0.015\%$ ) in the T3 groups. The highest nitrogen free extract ( $37 \pm 0.043\%$ ) was observed in the control group compared to the other treatment groups. There is a no significantly ( $p > 0.05$ ) difference among the treatment groups.

**TABLE 2** Proximate composition of different experimental feeds.

Treatment	Protein (%)	Lipid (%)	Moisture (%)	Ash (%)	Fibre (%)	Nitrogen free extract
Control	$25.07 \pm 0.015$	$8.46 \pm 0.026$	$11.16 \pm 0.015$	$14.25 \pm 0.010$	$4.32 \pm 0.015$	$36.92 \pm 0.047$
T1	$25.25 \pm 0.010$	$8.63 \pm 0.015$	$10.89 \pm 0.021$	$14.46 \pm 0.025$	$4.25 \pm 0.015$	$36.50 \pm 0.043$
T2	$25.30 \pm 0.015$	$8.76 \pm 0.025$	$10.77 \pm 0.020$	$14.65 \pm 0.015$	$4.17 \pm 0.015$	$36.33 \pm 0.010$
T3	$25.37 \pm 0.020$	$8.96 \pm 0.015$	$10.64 \pm 0.021$	$14.87 \pm 0.020$	$4.07 \pm 0.015$	$36.08 \pm 0.062$

Values are means  $\pm$  SD,  $n = 3$  per treatment group. T1, diet containing 0.5 g fish silage; T2, diet containing 1 g fish silage; T3, diet containing 1.5 g fish silage; control, diet without silage.

### 3.2 Haematological parameters of fish

The haematological parameters of fish observed at the end of the experiment are presented in Table 3. The parameters evaluated included white blood cells (WBC), red blood cells (RBC), haemoglobin (Hb), and haematocrit (HCT). The highest RBC count ( $3.81 \pm 0.0015 \times 10^6$ ) was recorded in the control group, while the lowest value ( $3.78 \pm 0.003 \times 10^6$ ) was observed in the T1 group. The highest WBC count ( $1.67 \pm 0.015 \times 10^4$ ) was observed in the T1 group, whereas the lowest value ( $1.57 \pm 0.02 \times 10^4$ ) occurred in the control group. The highest haemoglobin concentration ( $11.16 \pm 0.015$  g/dl) was recorded in the control group, while the lowest value ( $10.97 \pm 0.015$  g/dl) was observed in the T1 group. Similarly, the highest haematocrit value ( $27.55 \pm 0.04\%$ ) was observed in the

control group, whereas the lowest value ( $26.85 \pm 0.035\%$ ) was recorded in the T3 group. A significant difference was observed among all treatment groups ( $p < 0.05$ ).

### 3.3 The growth performance

The growth performance of common carp are represented in the Table 4 and Figure 1. There is significant difference in all parameters among the treatment groups ( $p < 0.05$ ). The highest length gain ( $5.96 \pm 0.05$  cm) was observed in the T3 group compared to the other treatment groups. The highest body weight gain ( $8.43 \pm 0.15$  g) was observed in the T2 group compared to the other treatment groups. The highest percentage weight gain ( $161.44 \pm 10.14\%$ ) was observed in the T3 group compared to the control groups. The highest specific growth rate

(0.46±0.01%) was observed in the T3 group compared to the other treatment groups. The highest food conversion ratio (1.91±0.47 g) was observed in the control group while the lowest value (1.26±0.07 g) was observed in T3 group compared to the other treatment groups. The highest protein efficiency ratio (3.03±0.18 g) was observed in the T3 while the lowest value (1.96±0.04 g) was

observed in control groups compared to the other treatment groups. The highest hepato-somatic index (0.05±0.00%) was observed in the T3 group and lowest (0.02±0.00%) in the control groups. The highest intestinal-somatic index (0.33±0.00%) was observed in the T3 group and whereas the lowest (0.25±0.00%) in the control groups.

**TABLE 3** Haematological parameters of common carp at the end of 60-day long experiment.

Treatment	RBC (×10 <sup>6</sup> )	WBC (×10 <sup>4</sup> )	Hb (gm/dl)	HCT (%)
Control	3.81±0.0015 <sup>a</sup>	1.64±0.010 <sup>b</sup>	11.16±0.015 <sup>a</sup>	27.55±0.040 <sup>a</sup>
T1	3.78±0.0030 <sup>d</sup>	1.67±0.015 <sup>a</sup>	10.97±0.015 <sup>d</sup>	27.39±0.041 <sup>b</sup>
T2	3.79±0.0020 <sup>c</sup>	1.62±0.015 <sup>b</sup>	11.03±0.015 <sup>c</sup>	26.85±0.035 <sup>d</sup>
T3	3.80±0.0026 <sup>b</sup>	1.57±0.020 <sup>c</sup>	11.09±0.025 <sup>b</sup>	26.97±0.015 <sup>c</sup>
F-values	64.39	24.32	59.62	275.24

WBC, white blood cells; EBC, red blood cells; Hb, haemoglobin; and HCT, haematocrit. Values are means ± SD, *n* = 3 per treatment group. Means in a column with different superscript letters differ significantly (*p* < 0.05), analyzed by one-way ANOVA and the DUNCAN test. T1, diet containing 0.5 g fish silage; T2, diet containing 1 g fish silage; T3, diet containing 1.5 g fish silage; control, diet without silage.

**TABLE 4** Growth performance of common carp during the experimental periods.

Treatment	Initial length (cm)	Final length (cm)	Initial Weight (g)	Final weight (g)	Length gain (cm)	Weight gain (g)	% weight gain (%)	Specific growth rate (%)	Food conversion ratio (g)	Protein efficiency ratio (g)	Hepato Somatic Index (%)	Intestinal Somatic Index (%)
Control	6.16±0.15 <sup>a</sup>	10.16±0.15 <sup>d</sup>	6.23±0.05	11.66±0.11 <sup>c</sup>	4.00±0.26 <sup>d</sup>	6.43±0.05 <sup>c</sup>	122.93±0.25 <sup>c</sup>	0.38±0.00 <sup>c</sup>	1.91±0.47 <sup>a</sup>	1.96±0.04 <sup>c</sup>	0.02±0.00 <sup>d</sup>	0.25±0.00 <sup>d</sup>
T1	6.33±0.05 <sup>a</sup>	10.76±0.05 <sup>c</sup>	6.33±0.15	12.66±0.50 <sup>b</sup>	4.43±0.15 <sup>c</sup>	7.33±0.35 <sup>b</sup>	137.45±2.70 <sup>b</sup>	0.41±0.00 <sup>b</sup>	1.71±0.14 <sup>b</sup>	2.33±0.05 <sup>b</sup>	0.03±0.00 <sup>c</sup>	0.27±0.01 <sup>c</sup>
T2	6.23±0.11 <sup>a</sup>	11.53±0.05 <sup>b</sup>	6.26±0.05	13.70±0.17 <sup>a</sup>	5.30±0.10 <sup>b</sup>	8.43±0.15 <sup>a</sup>	160.13±3.07 <sup>a</sup>	0.46±0.00 <sup>a</sup>	1.36±0.01 <sup>c</sup>	2.88±0.02 <sup>a</sup>	0.04±0.00 <sup>b</sup>	0.29±0.00 <sup>b</sup>
T3	6.36±0.15 <sup>a</sup>	12.33±0.15 <sup>a</sup>	6.33±0.15	13.93±0.15 <sup>a</sup>	5.96±0.05 <sup>a</sup>	6.60±0.30 <sup>a</sup>	161.44±10.14 <sup>a</sup>	0.46±0.01 <sup>a</sup>	1.26±0.07 <sup>d</sup>	3.03±0.18 <sup>a</sup>	0.05±0.00 <sup>a</sup>	0.33±0.00 <sup>a</sup>
F-values	1.596	198.83	1.395	40.62	87.11	51.44	34.79	39.46	105.99	73.70	115.57	58.39

Means in a column with different superscript letters differ significantly (*p* < 0.05), analyzed by one-way ANOVA and the DUNCAN test. T1, diet containing 0.5 g fish silage; T2, diet containing 1 g fish silage; T3, diet containing 1.5 g fish silage; control, diet without silage.

### 3.4 The carcass composition of fish body

The carcass composition of experimental fish was observed at end of the experiment are represented in Table 5. The nutritional composition of the experimental fish such as analyses of crude protein, lipid, moisture, ash, and nitrogen-free extract (NFE). The highest crude protein (60.29±0.04%) was observed in the T3 group whereas the lowest value (58.87±0.01%) was observed in the control groups. The highest crude lipid (13.78±0.01%) was observed in the T3 group whereas the lowest value (13.10±0.01%) was observed in the control groups. The highest moisture content (10.96±0.01%) was observed in the control group while the lowest (10.25±0.00%) was observed in the T3 groups. The highest crude ash (9.32±0.01%) was observed in the control group while the lowest (9.21±0.00%) was observed in the T3 groups. The highest nitrogen free extract (7.73±0.01%) was observed in the control group compared to the other treatment

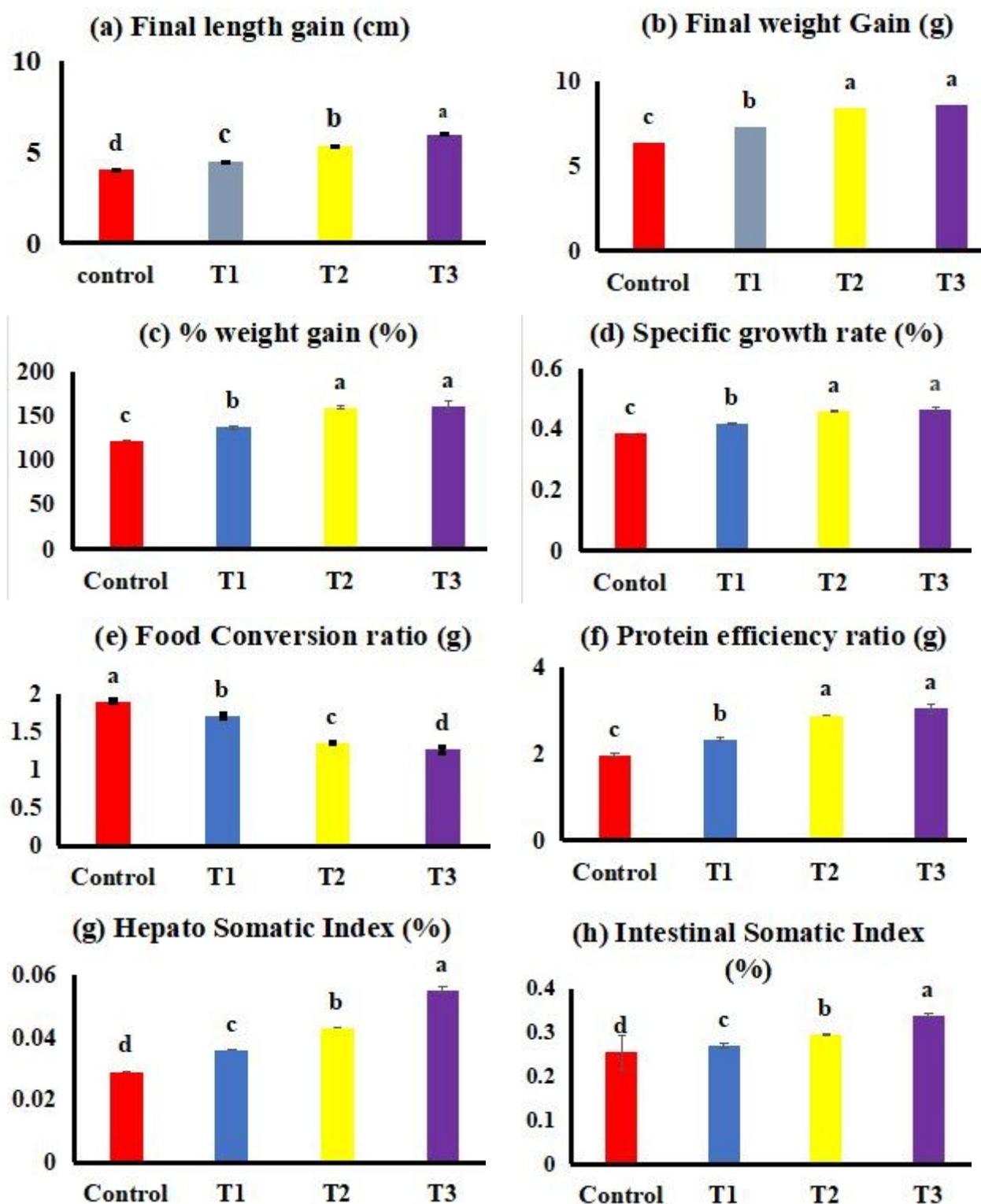
groups. There is a significantly (*p* < 0.05) difference in between all among the treatment groups.

### 3.5 The water quality parameters

The water quality parameters of experimental fish was observed during the experimental period are represented in Table 6. The acceptable range of water quality parameters was observed such as temperature, dissolved oxygen, pH, hardness and alkalinity. The highest water temperature (25.7–27.5°C) was observed in the T3 group whereas the lowest value (24.5–26.3°C) was observed in the control groups. The dissolved oxygen range from (5–6.5 mg/L) was observed during the experimental period in different treatment groups. The pH range from (7–8.5) was observed during the experimental period in different treatment groups. The water hardness range from (63–80 mg/L) was observed during the experimental period in different treatment groups. The water alkalinity range

from (70–80 mg/L) was observed during the experimental

period in different treatment groups.



**FIGURE 1** Growth performance of *Cyprinus carpio*; a. Final length gain; b. Final weight Gain; c. % weight gain; d. Specific growth rate; e. Food Conversion ratio; f. Protein efficiency ratio; g. Hepato-Somatic Index (HSI); h. Intestinal Somatic Index. \*data are presented as mean SE. Different superscripts indicate statically significant difference ( $p < 0.05$ ) among the experimental groups.

**TABLE 5** Carcass composition common carp after the feeding trial at the end of 60-day long experiment.

Treatment	Protein (%)	Lipid (%)	Moisture (%)	Ash (%)	Nitrogen free extract (%)
Control	58.87±0.01 <sup>d</sup>	13.10±0.01 <sup>d</sup>	10.96±0.01 <sup>a</sup>	9.32±0.01 <sup>a</sup>	7.73±0.01 <sup>a</sup>
T1	59.14±0.01 <sup>c</sup>	13.33±0.01 <sup>c</sup>	10.78±0.01 <sup>b</sup>	9.28±0.01 <sup>b</sup>	7.46±0.03 <sup>b</sup>
T2	59.66±0.01 <sup>b</sup>	13.56±0.01 <sup>b</sup>	10.56±0.02 <sup>c</sup>	9.23±0.01 <sup>c</sup>	6.97±0.03 <sup>c</sup>
T3	60.29±0.04 <sup>a</sup>	13.78±0.01 <sup>a</sup>	10.25±0.00 <sup>d</sup>	9.21±0.00 <sup>d</sup>	6.45±0.04 <sup>d</sup>
F-value	1937.77	1523.53	1395.61	57.40	946.98

Means in a column with different superscript letters differ significantly ( $p < 0.05$ ), analyzed by one-way ANOVA and the DUNCAN test. T1, diet containing 0.5 g fish silage; T2, diet containing 1 g fish silage; T3, diet containing 1.5 g fish silage; control, diet without silage.

**TABLE 6** The water quality parameters of common carp during the experimental period.

Treatment	Temperature (°C)	Dissolved oxygen (mg/L)	pH	Hardness (mg/L)	Alkalinity (mg/L)
Control	24–27	5–6.5	7–7.5	63–65	75.3–79.6
T1	24.5–26.3	5.5–6.5	7–7.8	65–70	76–80
T2	25.4–27.5	5–6.5	7.8–8.5	65–75	70–75
T3	25.7–27.5	5–6.5	7.5–8.2	75–80	75–79

T1, diet containing 0.5 g fish silage; T2, diet containing 1 g fish silage; T3, diet containing 1.5 g fish silage; control, diet without silage. Values are represented as a range of water quality parameters during the experimental periods.

## 4 | DISCUSSION

### 4.1 Proximate composition of feed

The proximate composition of the silage-based diet obtained in the present study revealed a balanced nutrient profile suitable for aquaculture feeds. The crude protein content (25.48±0.005%) falls within the optimum range reported for grow-out diets of many freshwater and carnivorous fish species, where dietary protein levels between 25–35% are considered adequate for supporting growth and maintenance (NRC 2011; Tacon and Metian 2015). The relatively high protein level indicates effective preservation of nitrogenous compounds during the silage process, as acid or fermented silage is known to minimize protein degradation while improving digestibility through partial hydrolysis of proteins into peptides and free amino acids (Raa and Gildberg 1982; Al Abri 2008). The lipid content of the diet (8.76%) is comparable with earlier reports on silage-based feeds, which generally range from 6–12% depending on the raw material used (Islam *et al.* 2021; Olsen and Toppe 2017). Lipids serve as a concentrated energy source and contribute essential fatty acids required for cell membrane integrity, hormone synthesis, and improved feed efficiency. Adequate lipid levels also help spare dietary protein for growth rather than energy metabolism, which is particularly beneficial in cost-effective aquafeed formulations (Glencross 2009). Moisture content was relatively low (10.77%), indicating good shelf stability of the silage-based diet. Low moisture levels are advantageous as they reduce microbial spoilage and improve storage life, which is often a limitation in wet silage products (FAO 2016). The ash content (14.65%) reflects a considerable mineral fraction, likely derived from bones and connective tissues present in the silage raw materials. Similar ash levels have been reported in

fish and poultry by-product silages and are considered beneficial as they supply essential macro- and micro-minerals such as calcium and phosphorus for skeletal development and metabolic functions in fish (Hardy 2010). The crude fibre content (4.17%) remained within acceptable limits for aquaculture feeds. Although fish have limited ability to digest fibre, moderate levels can aid gut motility and feed pellet integrity without adversely affecting nutrient utilization (Francis *et al.* 2001). The nitrogen-free extract (NFE) value of 36.14% suggests a substantial proportion of digestible carbohydrates, which can act as an additional energy source. Proper inclusion of carbohydrates helps reduce feed costs and further spares protein for growth, provided levels remain within species-specific tolerance limits (Wilson 1994).

### 4.2 Growth performance

The efficacy of incorporating fish silage into aquafeeds, aiming to evaluate its impact on the growth performance of various fish species. These studies have investigated the potential of fish silage as a sustainable alternative to traditional protein sources like fish meal and soybean meal. A study assessed the replacement of fish meal with co-dried fish silage (CFS) in the diets of common carp fingerlings. Four diets were formulated with 0%, 50%, 75%, and 100% replacement levels. The control group (0% replacement) exhibited the highest weight gain and specific growth rate (SGR), followed closely by the 50% replacement group. However, higher replacement levels (75% and 100%) led to a significant reduction in growth and feed utilization parameters. This suggests that up to 50% replacement of fish meal with CFS can be achieved without adversely affecting growth performance. Singh *et al.* (2018) evaluated the replacement of soybean meal with

fish silage at varying levels (15%, 30%, 45%, and 60%). The study found that a 45% replacement level resulted in the highest SGR ( $0.77 \pm 0.03$ ) and optimal feed conversion ratio (FCR) ( $2.22 \pm 0.08$ ) in common carp fingerlings. The fish silage can effectively replace a significant portion of plant-based protein sources in carp diets, enhancing growth performance. Bezuayehu *et al.* (2018) investigated the effects of fish silage inclusion in Nile tilapia diets revealed that incorporating up to 20% fish silage improved growth metrics such as weight gain and SGR. The study highlighted that fish silage possesses adequate nutritional value for tilapia fry at lower inclusion levels, making it a viable supplement in tilapia feed formulations. A study on rainbow trout examined the impact of replacing fish meal with fish silage at 25%, 50%, and 100% levels. The group fed with 50% fish silage exhibited the most significant weight gain, suggesting that partial replacement of fish meal with fish silage can positively influence growth performance in rainbow trout (Guzel *et al.* 2011). Research on pangas catfish fry demonstrated that complete replacement of fish meal with fish silage in the diet led to improved growth and feed utilization (Khan *et al.* 2021). Additionally, Khan *et al.* (2021) reported the positive effects on health indicators such as hematology, biochemistry, and antioxidant status, indicating that fish silage can serve as a cost-effective and health-promoting protein source in pangas catfish aquaculture.

#### 4.3 Haematological parameters

The present study investigated the effects of silage-based diets on the haematological parameters of common carp (*C. carpio*), with the aim of evaluating their physiological and health status in response to alternative protein sources. The findings revealed that the incorporation of silage, particularly fish or poultry by-product silage, significantly influenced several key haematological indices including haemoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC), and haematocrit (HCT). Consistent with earlier studies (Roslan *et al.* 2024), fish fed moderate inclusion levels (10–20%) of silage exhibited stable or slightly elevated haemoglobin and RBC values compared to the control group. This suggests that silage diets can support efficient oxygen transport and erythropoiesis, likely due to the presence of bioavailable iron and amino acids in the silage material. However, higher inclusion levels (>30%) were associated with a slight decline in these parameters, indicating possible stress or reduced nutrient digestibility at excessive inclusion rates (Kurhan *et al.* 2025). WBC counts were generally higher in fish fed silage-based diets, particularly those containing poultry by-product silage. This aligns with previous findings (Wirth *et al.* 2018) where an increase in WBC count was interpreted as a sign of enhanced immune activity or a mild inflammatory response. The elevated WBCs, particularly lymphocytes and monocytes,

may reflect the immune system's response to new or unfamiliar dietary antigens present in the silage. Moreover, the mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) remained within physiological limits, indicating no significant macrocytic or microcytic shifts in erythrocyte size or haemoglobin content. This suggests that the silage diets, when balanced with other essential nutrients, did not adversely affect erythropoiesis. Yang *et al.* (2019) reported that the fish silage-based diets, particularly when used in moderation, do not compromise the haematological health of common carp and may even support immune function. However, the data also suggest that excessive inclusion levels may pose risks to haematological stability, warranting further investigation into digestibility, palatability, and long-term effects on fish physiology.

#### 4.4 Carcass composition of common carp

The carcass and nutritional composition of fish is a direct reflection of dietary inputs and overall health, playing a significant role in determining market value and consumer preference. In the context of sustainable aquaculture, silage-based diets have emerged as promising alternatives to conventional fishmeal, particularly due to their affordability and potential to recycle waste materials. Numerous studies have investigated the impact of such diets on the proximate composition of common carp (*C. carpio*), focusing on key nutritional components including protein, lipid, moisture, and ash. Crude protein content in the carcass is a critical indicator of growth and muscle development. Previous findings suggest that fish fed diets containing moderate levels of silage (10–20%) generally exhibit similar or slightly improved carcass protein levels compared to those on conventional fishmeal diets (Aslan and Ozturk 2022). This indicates that silage, particularly from fish or poultry by-products, contains sufficient amino acids to support muscle protein synthesis. However, when silage inclusion exceeds optimal levels, protein deposition may decline, possibly due to imbalances in essential amino acids or reduced digestibility (Liao *et al.* 2025). Crude lipid content in the carcass often increases in fish fed silage-based diets, especially when the silage is derived from oily fish or contains high energy levels. Studies by Fan *et al.* (2021) reported enhanced lipid deposition in the muscle tissue of carp fed fermented fish offal silage. While moderate lipid accumulation contributes to improved flavor and energy reserves, excessive fat may affect flesh quality and consumer appeal, particularly in markets that prefer leaner fish. Moisture content in the carcass is inversely related to the concentrations of protein and lipid. Research indicates that carp fed diets with excessive silage inclusion (above 30%) often exhibit higher carcass moisture content (Magbanua and Ragaza 2024). This may be due to reduced nutrient absorption or imbalanced energy-protein ratios, leading to a leaner

body composition. High moisture levels can also impact fillet firmness and shelf life, which are important parameters for processing and storage. Ash content, reflecting the mineral composition of the carcass, is often influenced by the source and processing of the silage. Diets incorporating bone-in fish silage or poultry offal tend to elevate carcass ash levels (Al-Abri *et al.* 2014). Moderate increases in ash content can be beneficial, contributing essential minerals such as calcium and phosphorus, which are crucial for skeletal development. However, excessive mineral deposition may dilute other nutrients and affect the palatability and texture of fish flesh.

#### 4.5 Water quality parameters of fish

The water quality plays a pivotal role in the successful cultivation of common carp (*C. carpio*), influencing their physiological functions, growth, health, and survival. Among the most critical parameters are temperature, dissolved oxygen (DO), pH, water hardness, and alkalinity. These parameters interact with one another and collectively shape the aquatic environment that supports carp metabolism and productivity. Temperature is a key factor affecting fish metabolism, feeding rate, and enzyme activity. Previous studies have established that the optimal temperature range for common carp growth lies between 24°C and 28°C (Oyugi *et al.* 2012). Within this range, carp exhibit efficient feed conversion and higher growth rates. Temperatures below 20°C tend to slow metabolic processes and suppress immunity, while those above 30°C can induce thermal stress, reduce appetite, and impair physiological functions. Seasonal variations in temperature, therefore, need to be managed through site selection, pond depth, or shading strategies to maintain optimal thermal conditions. Dissolved oxygen (DO) is essential for respiration and aerobic metabolism. Although common carp are relatively tolerant of low DO levels compared to other fish species, prolonged exposure to levels below 3.0 mg L<sup>-1</sup> can lead to stress, poor feed utilization, and even mortality (Ali *et al.* 2022). Optimal DO levels for carp culture are generally above 5.0 mg L<sup>-1</sup> to support active metabolism and growth. Practices such as aeration, water exchange, and maintaining proper stocking densities are crucial to ensuring sufficient oxygen availability, especially during high biomass periods or warm temperatures when oxygen solubility decreases. pH directly affects fish physiology, ammonia toxicity, and nutrient availability. Common carp thrive in water with a pH between 6.5 and 8.5 (Menon *et al.* 2023). A stable pH within this range supports enzyme function, osmoregulation, and metabolic activities. Values below 6.0 or above 9.0 can cause stress, gill irritation, and impaired ion exchange. Sudden fluctuations in pH, often due to photosynthesis or decomposition of organic matter, should be avoided through proper water management and buffering. Water hardness, mainly governed by calcium and

magnesium ions, is important for bone formation, osmoregulation, and overall physiological balance. Studies have shown that a total hardness level between 75–150 mg L<sup>-1</sup> as CaCO<sub>3</sub> is suitable for carp culture (Molokwu and Okpokwasili 2002). Hard water also helps reduce the toxicity of certain harmful compounds such as heavy metals and ammonia. Water that is too soft (<50 mg L<sup>-1</sup>) may lead to poor bone development and increased sensitivity to environmental stressors. Alkalinity, which reflects the buffering capacity of water, is closely linked to pH stability. Optimal total alkalinity levels for common carp range from 75 to 200 mg L<sup>-1</sup> as CaCO<sub>3</sub> (Wurts 2002). Adequate alkalinity ensures resistance to rapid pH changes, especially in ponds with high photosynthetic activity. Inadequate buffering capacity can lead to daily pH swings that disrupt fish metabolism and immune response.

#### 5 | CONCLUSIONS

The 60-day feeding trial evaluating fish waste silage-based diets on the growth performance, feed utilization, haematology, and carcass composition of *C. carpio* demonstrated that the inclusion of silage in the diet positively influenced fish growth and overall health. Fish fed the silage-based diets exhibited superior performance in terms of length gain, weight gain, percentage weight gain, specific growth rate, protein efficiency ratio, hepatosomatic index, and intestinal somatic index. The highest growth metrics were recorded in the treatment groups, while the control group showed the least efficiency with a comparatively high feed conversion ratio. This indicates that fish waste silage enhances both nutrient absorption and metabolic efficiency in common carp. Haematological parameters, including white blood cells, red blood cells, hemoglobin, and hematocrit, remained within healthy ranges and showed improvement in the treatment groups, suggesting that the silage diets supported better physiological and immune responses. Carcass composition analysis also revealed stable protein, lipid, moisture, ash, and nitrogen-free extract levels across treatments, reflecting balanced nutrient assimilation. Water quality was consistently maintained within acceptable ranges throughout the experiment, ensuring that observed effects were attributable to dietary treatments rather than environmental fluctuations.

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## CONFLICT OF INTEREST

The author declares no conflict of interest.

## AUTHORS' CONTRIBUTION

Komal Prasad Sahu: Writing original draft, Methodology, Dushyant Kumar Damle: Writing review and editing, Visualization, Supervision, Investigation, Data curation, Conceptualization. Jham Lal: Writing review and editing, Formal analysis, Visualization, Validation, Supervision, Investigation, Data curation, Conceptualization. Harshavarthini M: Writing review and editing. Basant Singh: Writing review and editing. Vivek Kumar Thakur: Writing original draft, Methodology. Amisha Markam: Writing original draft, Methodology. Doman Nirmalkar: Writing original draft, Methodology. Ranjana Damle: Writing original draft, Methodology.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on a reasonable request from the corresponding author.

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